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Residues of some veterinary drugs in animals and foods

FAO
FOOD AND
NUTRITION
PAPER

41/8

Abamectin
Ceftiofur sodium
Chlortetracycline
Diclazuril
Doramectin
Fenbendazole,
febantel
and oxfendazole
Moxidectin
Oxytetracycline
Tetracycline

WORLD
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the
United
Nations

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Monographs prepared by the
forty-fifth meeting of the
Joint FAO/WHO Expert Committee
on Food Additives

Geneva, Switzerland, 6-15 June 1995

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Geneva, 6-15 June 1995

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ABBREVIATIONS USED IN THIS DOCUMENTS

ADI	-acceptable daily intake
AUC	-area under concentration-time curve
Av.	-average
b.i.d.	-twice a day
BP	-British Pharmacopoeia
Bq	-Becquerel (one disint/sec)
BST	-bovine somatotropin
bw, BW	-body weight
°C	-degrees Celcius
^{14}C	-radioactive Carbon-14
C_{max}	-maximum concentration
CAP	-chloramphenicol
µCi	-microcuries of radioactivity
cm^3	-cubic centimeter
conc	-concentration
CTC	-chlortetracycline
CV	-coefficient of variation
d	-day
DPM	-disintegration per minute
e.g.	-for example
EP	-European Pharmacopoeia
eq or EQ	-equivalents
F	-female
FDA	-Food and Drug Administration
g	-gram
µg	-microgram
GC	-gas chromatography
GI	-gastrointestinal
GLC	-gas-liquid chromatography
GLP	-Good Laboratory Practice
GVP	-Good Veterinary Practice
h	-hour
^3H	-tritium
HPLC	-high performance liquid chromatography
i.e.	-that is
i.m., IM	-intra muscular
i.m.i.	-intra muscular injection
i.p., IP	-intra peritoneal
i.v., IV	-intra venous
k_{el}	-rate constant
kg	-kilogram
L or l	-litre
LC	-liquid chromatography
LOD	-limit of detection
LOQ	-limit of quantitation
LSC	-liquid scintillation counting
M	-molar or mole
M	-male
max	-maximum
µm	-micrometer
mg	-milligram
min	-minute
ml	-millilitre
MR	-marker residue
MRL	-maximum residue limit
MRT	-mean residence time
MS	-mass spectrometry
n or No	-number
na	-not analyzed, assayed or available
nd, ND	-not detected
NER	-non extractable residues
ng	-nanogram
nm, NM	-not measured, if applicable
nm	-nanometer, if applicable
NMR	-nuclear magnetic resonance
NOEL	-no-observed-effect level
OTC	-oxytetracycline
ppb	-parts per billion
ppm	-parts per million
r	-regression coefficient
RIA	-radioimmunoassay
SA	-Specific Activity
s.c.	-subcutaneous
SD	-standard deviation
SEM	-standard error of mean
s.i.d.	-once a day
$t_{1/2}$	-half life
t_{max} or T_{max}	-time for maximum
TC	-tetracycline
TLC	-thin layer chromatography
TMS	-trimethyl silyl
TR	-total residues
TRA	-total radioactivity
TSD	-termionic specific detection
UD	-unchanged drug
USDA	-US Department of Agriculture
USP	-United States Pharmacopeia
UV	-ultraviolet
V_D	-volume of distribution
v/v	-volume/volume
wt	-weight
w/v	-weight/volume
WT	-withdrawal time
%	-per cent
>	-greater than
<	-less than
≤	-equal or less than

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INTRODUCTION

The Monographs on the residues of the eleven compounds contained in this volume were prepared by the forty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 6-15 June 1995. JECFA has evaluated veterinary drugs at previous meetings, including the 12th[1], 26th[2], 27th[3], 32nd[4], 34th[5], 36th[6], 38th[7], 40th[8], 42nd[9], and 43rd[10] meetings.

In response to a growing concern about mass-medication of food producing animals and the implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[11]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly-created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[12]. In response to these recommendations , the thirty-second JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, 43rd, and 45th meetings of JECFA were also dedicated exclusively to evaluation of veterinary drugs.

The eight session of the CCRVDF, held in Washington D.C. during June 1994, revised the priority list of veterinary drugs requiring evaluation. The drugs evaluated during the 45th meeting of JECFA included these compounds, except levamisole and triclabendazole.

The present volume contains summary monographs of the residue data on all of the eleven compounds on the agenda. From the six anthelminthic agents, febantel, fenbendazole and oxfendazole had been previously evaluated by the 38th meeting of JECFA. Abamectin, doramectin, and moxidectin had not been considered before.

From the four antimicrobial agents, oxytetracycline had previously been evaluated by the Committee at the 12th and 36th meetings. The remaining antimicrobial agents, ceftiofur, chlortetracycline and tetracycline had not been evaluated before.

The antiprotozoal agent, diclazuril, had not been considered before.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 45th meeting is included in Annex 1.

The assistance of the experts and FAO consultant in preparing these monographs is gratefully acknowledged.

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11. Residues of Veterinary Drugs in Foods, Report of a Joint FAO/WHO Consultation, Rome, 29 October - 5 November 1984. FAO Food and Nutrition Paper No. 32, 1985.

12. Report of the First Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Washington D.C., 27-31 October 1986.

ABAMECTIN

First draft prepared by
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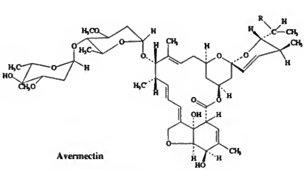
IDENTITY

Abamectin consists of two compounds differing from each other by only one methylene ($-CH_2-$) group. The two compounds are designated as avermectin-B_{1a} (or B_{1a}) and avermectin-B_{1b} (or B_{1b}). Abamectin contains no less than 80 % B_{1a} and no more than 20 % B_{1b}.

Chemical name:	Component B_{1a}: (2aE,4E,8E)-(5'S,6S,6'R,7S,11R,13S,15S,17aR,20R,20aR,20bS)-6'-[(S)-sec-butyl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-7-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside Component B_{1b}: (2aE,4E,8E)-(5'S,6S,6'R,7S,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-6'-isopropyl-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-7-yl 2,6-dideoxy-4-O-(2-6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside

Structural formula:



Avermectin

B_{1a}: $R = -CH_2-CH_3$
B_{1b}: $R = -CH_3$

CAS name and number:	5-O-Demethylavermectin A_{1a}, 65195-55-3 5-O-Demethyl-25-de(1-methylpropyl)-25(1-methylethyl)avermectin A_{1a}, 65195-56-4

Molecular formula:	$C_{48}H_{72}O_{14}$ (B_{1a} component); and $C_{47}H_{70}O_{14}$ (B_{1b} component)
Molecular Weight:	873.09 (B_{1a} component); and 859.06 (B_{1b} component)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Degree of impurity:	Avermectin B_{1a}, min. 80 % Avermectin B_{1a} + B_{1b}, min. 95 %
Appearance:	Off-white to slightly yellow, nonhygroscopic crystalline solid
Melting point:	150-155°C
Optical rotation:	$[\alpha]_D^{25^\circ} = 60^\circ$
Solubility:	Insoluble in water; freely soluble in acetone, chloroform or toluene; soluble in isopropanol; sparingly soluble in n-butanol, ethanol or methanol; slightly soluble in cyclohexane.
Partition (Distribution) coefficient:	9.9×10^3 determined in a n-octanol-aqueous buffer system, pH 7.
Ultraviolet absorbance spectrum:	Absorbance maxima at 237 nm, 245 nm, and a «shoulder» at 253 nm with respective log ϵ values of 4.48, 4.53 and 4.34 (in methanol).

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Avermectin has been developed for use in controlling various phytophagous pests in field crops and citrus (Putter et al., 1981) and fire ants (Lofgren et al., 1982).

Avermectin is also a potent endo- and ectoparasitic agent with a broad spectrum of activity in several animal species. It is a fermentation product produced by the actinomycete *Streptomycetes avermitilis*. (Burg et al., 1979).

PHARMACOKINETICS AND METABOLISM STUDIES

Rat

A study was carried out to evaluate the tissue distribution and elimination of tritium- and carbon-14-labeled avermectin B_{1a} after oral administration to male and female rats (Maynard et al., 1986). Sixty-two male and sixty-two female rats were divided into six groups each. The dosing of the groups were as follows : Group 1, a single high dose (1.4 mg/kg bw) of tritium-labeled avermectin B_{1a}; Group 2, a single low dose (0.14 mg/kg bw) of tritium-labeled avermectin B_{1a}; Group 3, fourteen daily low doses (0.14 mg/kg) of unlabeled avermectin B_{1a} followed by a single low dose of tritium-labeled avermectin B_{1a}; Group 4, a single high dose (1.4 mg/kg) of a mixture of tritium- and carbon-14 labeled avermectin B_{1a}; Group 5, a single dose of vehicle only to serve as control for groups 1, 2, and 4; and Group 6, fifteen daily doses of vehicle only to serve as control for Group 3. The control groups were sacrificed seven days after the last (or single) dose, while rats from the treated groups were sacrificed on days 1, 2, 4 and 7 after dosing. Samples were assayed for total radioactivity and also measured by reverse isotope dilution assay (RIDA) and reverse phase high performance liquid chromatography

(RP-HPLC).

Total Radioactivity

Since plasma or bile data were not available, no absolute measure of absorption is provided. High concentrations of radioactivity in faeces suggest either poor absorption or elimination through bile. 90% of the dose was recovered of which 70% in faeces.

The residue levels measured as total radioactivity were dose dependent as the residues in Groups 1 and 4 (1.4 mg/kg dose) were roughly ten-fold greater than those in Groups 2 and 3 (0.14 mg/kg dose). In contrast, the depletion rates were dose-independent, as to the half lives of Groups 1 and 4 compared to those of Groups 2 and 3 in the same tissues. There was no great difference in depletion half-lives among the tissues as most of the calculated half-lives were within the range of 1.2 ± 0.3 days.

Among liver, kidney, muscle, and fat, the fat had the highest residue levels, though kidney levels at one and two days in some groups were comparable to the fat levels. In most cases, kidney residue levels were higher than the levels in liver.

The tritiated avermectin B_{1a} residue levels and depletion rates in Groups 1 and 4 were of comparable value to the same parameters of the carbon-14 labeled avermectin B_{1a} in Group 4.

There was no effect on either the residue levels or depletion rates from fourteen daily 0.14 mg/kg doses of unlabeled avermectin B_{1a} prior to one 0.14 mg/kg dose of tritiated avermectin B_{1a} (Group 3) relative to a single 0.14 mg/kg dose of tritiated avermectin B_{1a} (Group 2).

The total residue levels in liver, kidney, muscle, and fat of the females of each group were generally higher than residue levels in the corresponding tissues of the males. However, the depletion rates were similar for residues in the tissues of the male and female rats.

The female rats eliminated 0.3 to 0.6% of the dose in urine, as compared to 0.8 to 1.1% of the males. The females excreted 68.7 to 76.7% of the dose in feces, as compared to 70.1 to 81.6% of the males.

RIDA

From data on the levels of avermectin in tissues as measured by RIDA the following results were observed: (a) the amount avermectin B_{1a} of the total residues was not dose dependent; (b) the half-life for avermectin B_{1a} in male tissues (0.6-1 day) was slightly less than in female tissues (0.9-1 day); (c) the amount of avermectin B_{1a} of the total residues was similar for both tritium and carbon-14 labeled avermectin B_{1a}; (d) the amount of avermectin B_{1a} of the total residues was lower in male than female tissues; (e) the amount of avermectin B_{1a} of the total residues was only slightly affected by the pre-treatment with avermectin B_{1a}.

RP-HPLC

The tissue residues were characterized by RP-HPLC radio-activity profiles. In addition to unchanged avermectin B_{1a}, two metabolites were observed. Identification of these two metabolites was accomplished by retention time comparison to standard compounds and by co-chromatography with these standard compounds. These metabolites that were also isolated from a rat liver microsomal incubation of avermectin B_{1a} and were identified as 24-hydroxymethyl-avermectin B_{1a} (24-OHMe-B_{1a}) and 3"-desmethyl avermectin B_{1a} (3"-DM-B_{1a}). Most of the residues in muscle, fat and organ tissues of rat were identified as avermectin B_{1a}, 24-OHMe-B_{1a}, and 3"-DM-B_{1a}.

The RP-HPLC radioactivity profiles of tissue extracts from rats (Group 4) dosed with both tritium and carbon-14 labeled avermectin B_{1a} were investigated. Both ^{3}H-b_{1a} and ^{14}C-B_{1a} had identical profiles with all radio-active peaks having a constant ^{3}H/^{14}C ratio and the same percentage of recovered radioactivity. Therefore, the metabolism of both ^{3}H- and ^{14}C-avermectin B_{1a} by rats was identical demonstrating that the use of tritium labeled avermectin B_{1a} is appropriate for animal metabolism studies.

In males, avermectin B_{1a} accounts for 50% of the total residues in kidney, liver and muscle at one day post dosing. At 4 days, it represents 35%, and at 7 days less than 10%. In females, the average values were respectively 55-74% at one day, 45% at 4 days, and 16-28% at 7 days. These results indicate that the metabolism of avermectin B_{1a} was higher in males than in females. A non-polar fraction has been investigated in fat as it accounts for 5-8 % of the total residues at one day post dosing and 41-64% at 7 days. The results obtained indicated that this fraction contains conjugates of 24-OHMe-B_{1a} and 3"-DM-B_{1a} metabolites.

TISSUES RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies in cattle

Cattle

Twelve Angus steers were given a subcutaneous injection of 0.3 mg tritium-labeled abamectin /kg body weight. The animals were slaughtered at 7, 14, 21, and 35 days post-dose for collection of tissues. Tissues were assayed for total radioactive residues and also by RIDA and RP-HPLC.

Total radioactivity

The mean plasma concentration of radioactivity peaked at 95 μg abamectin equivalents/l (range 34 to 162 μg/l) at 1-2 days post administration and declined with a half life of 4.7 days. The mean plasma concentration declined to 1 μg/l at day 35.

Abamectin is eliminated mainly in faeces. About 50% of the dose was recovered within 7 days in faeces and 1-2% in urine.

The average residue levels for the tissues are summarized in Table 1.

The limit of detection was respectively 0.7 μg/kg for muscle, 4 μg/kg for injection site, 0.3 μg/kg for liver, 1 μg/kg for kidney and 3 μg/kg for fat. (Lu et al., 1984).

Table 1. Total residues as abamectin equivalents ± SD (μg/kg) found in cattle injected subcutaneously with abamectin at 0.3 mg/kg bw

Days Post Dose	Muscle	Injection Site Muscle	Liver	Kidney	Fat
7	42 ± 10	2022±1720	619 ± 190	143 ± 37	444±110
14	10 ± 8	537 ± 61	168 ± 95	36 ± 8	130±76
21	4 ± 1	59 ± 87	61 ± 33	11 ± 5	63±22
35	1 ± 1	< 4	9 ± 6	2 ± 1	35±10

The depletion rates of residues in kidney, liver, and muscle between 7 and 35 days were all similar. Of the edible tissues, the liver had the highest residue levels at 7 and 14 days. By 21 days, the average liver residue levels had dropped to less than the average fat residue levels. The corresponding half-lives for residue depletion in kidney, liver and muscle were 5.7, 4.6, and 5.6 days, respectively.

The residue half-life in fat, 8.1 day, was longer than that in the other tissues. The muscle injection site had the highest residue level at 7 days but had also the highest depletion rate, with a half-life of 3.3 days.

RIDA

Liver and fat tissues from steers slaughtered 7, 14, and 21 days post dosing were analyzed for unaltered drug by RIDA. Results indicate that B_{1a} accounted for 34-61% of the total tissue residues in all liver samples studied. The minor drug component, B_{1b}, contributed to only 0.9-4.8% of the total residues in these tissue samples. The unaltered B_{1a} in the fat tissue accounted for 52, 40 and 25% of the total tissue residues at 7, 14, and 21 days post dose, respectively (Table 2). Similar to that in the liver tissue the B_{1b} component contributed to only 0.5-5.2% of the total residues in these fat samples. The RIDA results indicate that the unaltered drug should be a satisfactory marker substance in both the liver and fat tissue and the former should be a satisfactory target tissue for metabolism studies. (Lu et al., 1985).

Table 2. **Total residue concentrations (µg/kg) and % of B_{1a} in liver and fat of cattle dosed subcutanously with 5-^{3}H-abamectin at 0.3 mg/kg bw**

Days Post Dose	Liver		Fat	
	TR	% B_{1a}	TR	% B_{1a}
7	616	61	462	47
	811	34	544	58
14	275	41	216	41
	139	48	105	39
21	62	44	62	24
	94	41	85	26

RP - HPLC

In addition to the assay of the unaltered drug, the profile and composition of the total radioactive residues from the liver tissue of a 14 days post dose steer was studied by solvent fractionation and HPLC. The liver residues were classified on the basis of polarity by RP-HPLC elution. A group of polar metabolites accounting for 22% of the total residue is present in this sample among which the major metabolite was identified as 24-hydroxymethyl-B_{1a} by chromatographic comparison with an *in vitro* metabolite sample generated by rat liver microsomal incubation of B_{1a}.

Similar residue profile studies were carried out with a fat tissue sample from a steer slaughtered 21 days after dosing. Other than the unaltered drug, the major portion of the radioactivity resided in two groups of nonpolar metabolites of which the major one consisted of at least 7 compounds and accounted for 51% of the total tissue residues. Upon treatment with cholesterol esterase, these nonpolar metabolites gave one major polar product which was identified as 24-hydroxymethyl-B_{1a} by mix-sample chromatography with unlabeled *in vitro* metabolite samples.

For the purpose of comparison, residues from composite liver and fat tissue of rats dosed orally with 5-^{3}H-B_{1a} were studied similarly to those from the steer. Examination of the residue profile and composition shows that essentially the same pattern of metabolism exists in the two species. (Lu et al., 1985).

Other Residue Depletion Studies (with Unlabeled Drug)

Cattle

Thirty nine male castrates and 39 female cattle, aged approximately 18 months and weighing between 292 and 388 kg, were allocated to one of seven treatment groups: Group 1 untreated controls (3 males and 3 females), and Groups 2 to 7 each consisting of six males and six females treated subcutaneously with 0,2 mg abamectin/kg bw. The animals were sacrificed on days 21, 28, 35, 42, 49, and 56, respectively, and assays were carried out using an HPLC/fluorescence method having a detection limit of 1 µg/kg.

By 42 days post-treatment the mean tissue residues of avermectin B_{1a} had depleted to 2 µg/kg or less, for injection site from 5,200 µg/kg (range 11 to 33,000) at 21 days to 2 µg/kg (range 0 to 22), for liver from

53 μg/kg (14 to 88) to 2 μg/kg (0 to 6), for fat from 78 μg/kg (17 to 200) to 2 μg/kg (0 to 6), for muscle from 6 μg/kg (1 to 9) to below the limit of detection-1 μg/kg (0 to 1), and for kidney, from 13 μg/kg (3 to 27) to 1 μg/kg (0 to 2). Mean residues in liver and injection site had depleted to below the detection limit by 49 days post-treatment. Other tissues at this time point were not assayed. The results are summarized in Table 3. (D.G. Baggot et al,. 1992).

Table 3. Avermectin B_{1a} residues (μg/kg) found in cattle injected subcutaneously with abamectin at 0.2 mg/kg bw

Days Post Dose	Muscle	Injection Site	Liver	Kidney	Fat
21	6	5200	53	13	78
28	2	2000	14	4	13
35	1	550	9	2	5
42	<1	2	2	1	2
49	NA	<1	<1	NA	NA

NA: Not Assayed

Six groups of 5 cattle each were dosed subcutaneously at approximately 0.3 mg/kg bw. Selected liver, fat, muscle, injection site and kidney tissues were assayed from animals sacrificed at 5, 10, 15, 20, 25 and 35 days post injection. The assays were carried out with an HPLC fluorescence method having a detection limit of 1 μg/kg. The injection site tissue residues averaged 21 μg/kg avermectin B_{1a} for animals sacrificed at 5 days post dose and depleted to an average of 0.57 μg/kg at 35 days post dose. The liver tissue residues depleted from a mean of 310 μg/kg at 5 days post dose to a mean of 16 μg/kg at day 35. The fat tissue residues paralleled the liver residues and averaged 320 and 12 μg/kg at 5 and 35 days, respectively. The muscle tissue contained residues averaging 27 μg/kg at 5 days post dose and depleted to an average of 2 μg/kg at 35 days post dosing. The kidney tissue residues depleted from an average of 36 μg/kg at 15 days to an average of 6 μg/kg avermectin B_{1a} by 35 days post dose. These results are summarized in Table 4. (T.A. Wehner et al., 1991).

Table 4. Avermectin B_{1a} residues (μg/kg) found in cattle injected subcutaneously with abamectin at 0.3 mg/kg bw

Days Post Dose	Muscle	Inj. Site Muscle	Liver	Kidney	Fat
5	27	21000	310	NA	320
10	NA	3300	210	NA	130
15	8	5400	110	36	73
20	3	800	30	9	30
25	< 1	1600	7	2	4
35	2	570	16	6	12

NA: Not Assayed

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The method proposed to control abamectin residues is an HPLC fluorescence assay of avermectin B_{1a}.

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(Wehner et al., 1991b).

The basis of the method is the isolation of the marker residue by solvent extraction followed by preparation of a fluorescent derivative. The method is applicable to muscle, liver, kidney and fat. Its limit of quantification is 5 μg/kg and limit of detection 1-2 μg/kg.

At about 2.5 μg/kg level, improvement in the method and greater separation from control tissue constituents can be achieved by adding an additional clean up step. At the 1 μg/kg level satisfactory assay values can only be achieved by either major revisions of the method or, slight modification of the HPLC system used. Below 1 μg/kg the HPLC system is not sensitive enough when 5-gram samples are used.

The average recoveries of avermectin B_{1a} for the different tissues were in the range of 70-90% and the coefficient of variation below 15%. This method is specific to avermectin B_{1a}.

APPRAISAL

In reaching its decision on the MRLs for abamectin, the Committee took into account the following:

- An ADI of 0-0.2 μg per kg of body weight has been established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1994. This would result in a maximum daily intake of residues of 12 μg for a 60-kg person;

- Liver and fat are considered as the appropriate target tissues;

- Abamectin, used as veterinary drug, is only intended for use in beef cattle;

- In fat tissue, abamectin does not lead to bound residues and less than 15% in liver;

- Avermectin B_{1a} is considered as an appropriate marker residue;

- Avermectin B_{1a} accounts for 42% of the total residues in liver and 25% for fat at 21 days post-dosing; and

- The limit of quantification of the proposed analytical method is 5 μg/kg.

The Committee noted that the 1992 JMPR evaluated abamectin residues resulting from pesticide use. The ADI of 0-0.2 μg/kg of body weight was established by JMPR using a safety factor of 500 to account for the concern of the delta-8,9 isomer of B_{1a} which does not occur with use as a veterinary drug.

MRLs have been recommended by JMPR for some vegetables and fruits, and for cattle and goat meat and milk to take into account possible contamination of these food commodities resulting from the ingestion of vegetables and fruits treated with abamectin by these animals.

According to the food basket that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) uses, the daily intake of residues, resulting from the following MRLs recommended by JMPR for these two animal species, 10 μg/kg for muscle, 50 and 100 μg/kg for offal in cattle and goat, respectively, and 5 μg/kg for milk, would significantly exceed the ADI of 12 μg/person.

In order to comply with the ADI, the Joint FAO/WHO Expert Committee on Food Additives would have to recommend MRLs for cattle muscle, liver, kidney and fat different from those recommended by the Joint FAO/WHO Meeting on Pesticide Residues.

Therefore, the Committee did not recommend MRLs for abamectin used as veterinary drug and recommended that consultations be held between representatives of JECFA and JMPR to resolve the ADI and MRL issues.

(Editor's note: Consultations were held 15 September 1995. Subsequently, JMPR at its 1995 meeting increased the ADI for abamectin to 0-1 μg per kg of body weight for uses in animal health. MRLs for abamectin will be reconsidered by JECFA at its 47th meeting, scheduled to be held in Rome, June 1996.)

REFERENCE

Baggot, D.G. et al. (1992). A study to determine avermectin B_{1a} residues in liver, kidney, fat, muscle and injection site tissue from cattle dosed subcutaneously with abamectin (MK 0936) at 1 ml 1 % formulation per 50 kg body weight. Merck Sharp and Dohme Research Laboratories report

Lu, A.Y.H. et al. (1984). Tissue depletion of tritium-labeled MK 0936 in cattle dosed subcutaneously (Ca-189). Merck Sharp and Dohme Research Laboratories report

Lu, A.Y.H. et al. (1985). Metabolic disposition of 5-^{3}H-MK 0936 in edible tissues of steers dosed subcutaneously at 0.3 mg/kg. Merck Sharp and Dohme Research Laboratories report

Maynard, M.S. et al. (1986). The metabolism of avermectin B_{1a} in rats. Merck Sharp and Dohme Research Laboratories report

Wehner, T.A. et al. (1991a). A study to determine Avermectin B_{1a} residues in liver, kidney, fat, injection site and muscle tissue from cattle dosed subcutaneously with MK 0936. Merck Sharp and Dohme Research Laboratories report

Wehner, T.A. et al. (1991b). HPLC fluorescence assay method for avermectin B_{1a} (MK 0936) in bovine tissue. Merck Sharp and Dohme Research Laboratories report

CEFTIOFUR

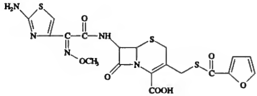
First draft prepared by
Dr. J.D. MacNeil
Agriculture & Agri-Food Canada
Health of Animals Laboratory
Saskatoon, Canada

IDENTITY

Chemical names: Ceftiofur; IUPAC name: [6R-[6α,7β(Z)]]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Synonyms: Ceftiofur sodium, Na ceftiofur, Naxcel, Excenel, CM 31-916, U64279E (Product Trade Names)

Structural formula:



Ceftiofur

Molecular formula: $C_{19}H_{17}N_5O_7S_3$

Molecular weight: 523.55

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Melting point: Degrades on heating (as sodium salt, at 190 to 300°).

Solubility: Freely soluble in water as sodium salt (>400 mg/mL in buffered solutions above pH 5.5); found in solutions as free acid at pH <3.2 with solubility of approximately 0.25 mg/mL. The sodium salt is slightly soluble in organic solvents such as iso-octane (0.006 mg/mL) and chloroform (0.008 mg/mL) and solubility increases for such solvents as ethyl acetate (0.038 mg/mL), acetonitrile (0.072 mg/mL), toluene (0.078) and methylene chloride (0.114 mg/mL) to higher values for tetrahydrofuran (0.2 mg/mL) and n-butyl chloride (0.76 mg/mL).

Optical rotation: As sodium salt, 67° for a 1% w/v aqueous solution at 589 nm.

Other properties: Ceftiofur sodium is hygroscopic. X-ray diffraction patterns indicate an amorphous structure.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Ceftiofur is a third-generation cephalosporin antibiotic developed exclusively for veterinary use. It is available commercially as the sodium salt and exhibits a broad spectrum of activity against both gram-positive and gram-negative bacteria, including β-lactamase-producing strains. It is also active against some anaerobic bacteria. Ceftiofur is bactericidal *in vitro*, inhibiting cell wall synthesis. It is rapidly metabolized *in vivo* to desfuroylceftiofur, which is also microbiologically active. Ceftiofur sodium is off-white to tan in colour and the formulated product may be stored frozen for up to 8 weeks without loss of potency. Storage of the unreconstituted product at a refrigerator temperature of 2 to 8°C is recommended. The reconstituted product is stable under these conditions for up to 7 days, or for 12 hours at room temperature. The product should be protected from light during storage as it is light sensitive. Direct contact with the skin and mucous membranes should be avoided by humans handling the drug to minimize the possibility of a reaction in sensitized individuals. Ceftiofur has not been previously reviewed by the Committee.

Dosage

Ceftiofur sodium is formulated as a dry, lyophilized sterile powder which is re-constituted in sterile or bacteriostatic water for injection. It is available in 1- and 4-gram vials containing ceftiofur sodium equivalent to 50 mg/mL ceftiofur. The recommended dose for cattle is 1.0 to 2.2 mg ceftiofur free acid equivalents (CFAE) per kilogram of body weight, repeated at 24-hour intervals on three consecutive days. When necessary, the treatment may be extended to days 4 and 5. For swine, the recommended dose is 3.0 - 5.0 mg ceftiofur (CFAE) per kilogram of body weight at 24 hour intervals on three successive days. Ceftiofur formulated product is intended only for administration by intramuscular injection in cattle and swine.

METABOLISM AND PHARMACOKINETICS

General

Ceftiofur has been observed *in vivo* to have a more complex metabolism than other cephalosporins, primarily due to the cleavage of its thioester bond, which yields desfuroylceftiofur (DFC) and furoic acid. Studies comparing the metabolism of ceftiofur by the S-9 microsomal enzyme fraction from liver and kidney tissues of various species (rats, pigs, cattle, chickens) demonstrated that ceftiofur was converted *in vitro* first to DFC, with the primary metabolite in kidney being desfuroylceftiofur cysteine disulfide (DCD) and, in liver, being DFC and 3,3'-desfuroylceftiofur disulfide dimer (3,3'-DFD) (Gilbertson *et al*, (1990). Qualitatively, results observed were the same for all species, but metabolism was slower in S-9 fractions from liver and kidney of rats than for the other species tested. Highest concentrations of 3,3'-DFD were observed in chicken liver and kidney S-9 fractions in the *in vitro* studies, while lowest concentrations of this compound were found in rat tissue fractions.

Rat

Following a single oral dose of 200 mg/kg BW ^{14}C-ceftiofur sodium salt, a similar pattern of metabolic breakdown was observed in rats to that described below for cattle receiving the drug by intramuscular injection (Jaglan & Arnold, 1986a,b). The initial metabolite was identified as DFC. Urine contained desfuroylceftiofur thiolactone and DCD (Jaglan & Roof, 1989). Oral administration of ^{14}C-ceftiofur to rats at a rate of 800 mg/kg BW/day for 5 successive days confirmed that DFC was the central metabolite and demonstrated that a similar pattern of metabolites was found in the livers and kidneys of the rats as that observed in tissues of cattle receiving an intramuscular injection of 44 mg/kg BW (Jaglan *et al*, 1987b). Additional polar metabolites were produced in rats that were not found in studies with cattle and sex related differences in these metabolites were observed only in rats. The primary urinary metabolite in rats was ceftiofur sulfoxide cysteine thioester, shown to be produced by enteric metabolism after oral dosing (Jaglan & Arnold, 1987a). Additional studies using improved LC separations for the metabolites confirmed that the thioester was the primary urinary metabolite in rats, in both 8-hour and 24-hour urine samples after oral dosing (Jaglan & Roof, 1990).

Ceftiofur sulfoxide cysteine conjugate was not detected at low oral doses of 15 mg/kg BW in rats, but was found at dosage rates of 100 - 1000 mg/kg BW (Jaglan, 1987). This compound was not found in the studies conducted with S-9 fractions of liver and kidney from rats and other species (Gilbertson *et al*, 1990), suggesting that this compound is formed uniquely in the gastrointestinal tract of rats. Intramuscular injection of rats with ceftiofur sodium salt produced the same metabolic profile as was seen for this treatment in cattle, with the exception that urine from the rats contained 4.4-21% unmetabolized ceftiofur (Jaglan & Arnold, 1987).

In rats, the primary metabolite in plasma, DFC, was present in a bound form, while DFC was in a free form in cattle plasma (Jaglan & Arnold, 1986b). Further research in rats injected intramuscularly with ^{14}C-ceftiofur sodium demonstrated that, in serum, covalent binding of DFC occurred primarily to albumin and α-1-antitrypsin through the formation of a thioester bond at the HS-group (Jaglan *et al*, 1991). Oral treatment with either the sodium or the hydrochloride salt of ceftiofur produced the same metabolic results (Jaglan *et al*, 1987a).

Incubation of ^{14}C-ceftiofur (119 mg/l) for 15 minutes with cofactor-augmented media containing the S-9 fraction of rat liver microsomal supernatants from Arochlor-1254 induced Fischer 344 rats resulted in 98.5% conversion to DFC. When the initial concentration of ceftiofur was raised to 857 mg/L, 52.5% conversion to DFC was observed at 15 minutes, with complete conversion in 60 minutes (Jaglan *et al*, 1987c).

Cattle

In an early study of the distribution and elimination of ^{14}C-ceftiofur in cattle (Krzeminski *et al*, 1985), LC analyses demonstrated the presence of two apparent metabolites ("C1", "C") in plasma. At one hour post-treatment, C1 reached a maximum concentration, but was not detected at 16 hours after treatment. Further work indicated that the compound designated as "C" in the report was the metabolite and that "C1" was formed on exposure of "C" to trifluoroacetic acid used in sample dilution prior to storage. Two major radioactive peaks, as well as several minor ones, were found in urine samples. Subsequent studies described above comparing metabolism in the rat and the bovine provided identification of the major urinary metabolites and demonstrated that 3,3'-DFD was a major urinary metabolite in cattle (Jaglan & Arnold, 1987b; Jaglan, Kubicek & Gilbertson, 1987). The proposed metabolic pathway for cattle which receive ceftiofur by intramuscular injection is shown in Figure 1. This pathway is also considered to be representative for other mammalian species studied to date for intramuscular administration.

Analyses were conducted on samples collected following treatment of 6 lactating dairy cattle with 5 consecutive intramuscular injections (24-hour intervals) of 2.29 mg ^{14}C-ceftiofur sodium/kg BW (Jaglan *et al*, 1989a). It was observed that most of the ceftiofur was eliminated in the urine (62.8 ± 7.6%) and faecal matter (35.7 ± 9.7%) within 24 hours of treatment. Only 0.15 ± 0.03% of the ceftiofur was accounted for as residues in milk. Tissues collected from the animals at slaughter 5 days after the last injection again demonstrated, on analysis, that the highest residues were present in kidney (2.50 ± 0.67 mg/kg). Residues in liver were 0.37 ± 0.03 mgk/g, while those in fat and muscle were < 0.1 mg/kg. Maximum concentrations of ceftiofur were in blood samples taken 1 - 2 hours after each treatment (5.1 - 12.1 μg/mL). Residues were essentially in the plasma fraction and were essentially bound to macromolecules, as observed in experiments with rats and pigs. The two main metabolites found in urine samples collected 6 hours after the last treatment were 3,3'-desfuroylceftiofur disulfide (dimer) and DCD.

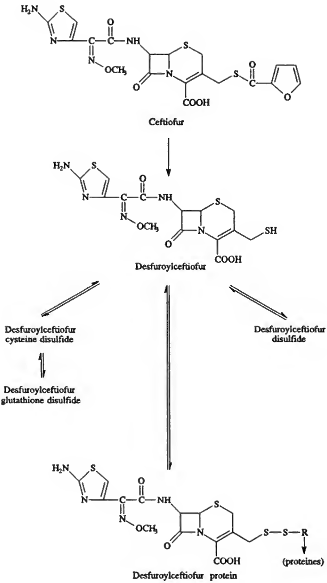
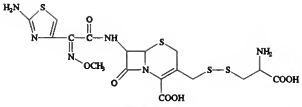
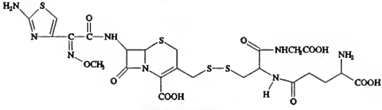

Ceftiofur
Desfuroylceftiofur
Desfuroylceftiofur cysteine disulfide
Desfuroylceftiofur glutathione disulfide
Desfuroylceftiofur disulfide
(proteines)
Desfuroylceftiofur protein

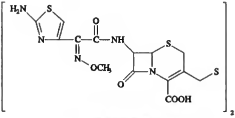
Figure 1. Proposed metabolism for ceftiofur in cattle.



Desfuroylceftiofur
cysteine disulfide



Desfuroylceftiofur
glutathione disufide



Desfuroylceftiofur
disulfide

Figure 2. **Proposed metabolites in cattle**

Residues in urine were about 10 μg/mL at 36 hours following the last injection and gave a positive result when assayed with the Live Animal Swab Test (LAST).

The half-life for ceftiofur sodium administered to dairy cattle has been calculated to be 212 minutes for intramuscular injection and 217 minutes for intravenous injection (Soback *et al*, 1989). This study demonstrated good absorption of the drug and a high estimated bioavailability, with no active residues detected in any milk samples collected (2, 4, 6 and 8 hours after drug administration, bioassay with *B. stearothermophilus* with detection limit of 0.1 mg/L).

Table 1. **Pharmacokinetics of ceftiofur in the bovine.**

Variables	Calves[a]	Calves[a]	Lactating cows[b]	Calves[c]
Dose (mg/kg)	1.0	1.0	2.0	2.2
Route of Administration	IM	IV	IV	IM
Method of Assay	HPLC-DCA[d]	HPLC-DCA	Bioassay	Bioassay
C_{max}(μg/mL)	4.12±0.84	7.09±1.59	ND[e]	8.78±2.47
t_{max}(h)	0.75±0.27	0.30±0.59	ND	1.82±0.89
AUCα(μg.h/mL)	34.3±6.29	35.9±11.6	27.2±5.42	66.2±21.6
$t_{½}$(h)	9.65±1.97	8.63±1.28	3.60±0.76	3.58±0.70
MRT(h)	11.4±1.75	10.3±1.72	5.07±1.10	ND
Vd_{ss}(L/kg)	ND	ND	0.39±0.16	ND
Cl_B(mL/min/kg)	ND	ND	1.27±0.26	ND

[a] Banting *et al*, 1989b.
[b] Soback *et al*, 1989.
[c] Halstead, 1990.
[d] HPLC-DCA refers to total desfuroylceftiofur, assayed as desfuroylceftiofur acetamide.
[e] ND means "not detected".

Data reported in a recent study where calves received ^{14}C-ceftiofur sodium intravenously at doses of 0.55, 2.2 and 8.8 mg CFAE/kg BW were consistent with those presented in the above table and demonstrated a proportional relationship between peak plasma concentrations and dosage rate (Brown *et al*, 1993). Based on the data for urinary excretion, there was also no indication of saturation of excretory pathways at the dosages tested. As this report was only available in draft form at the time of this evaluation, actual data from the study have not been included.

The distribution of ^{14}C-ceftiofur sodium was also studied in calves (94.0 - 136.4 kg), which each received three intramuscular injections of 2.2 mg/kg BW at 24-hour intervals (Johnson *et al*, 1985a), following which calves were sacrificed at 8 hours and 3, 21 and 39 days, respectively, and samples were analyzed by total radioactivity. Maximum residue levels (5 - 8.5 mg/L) in blood were at 0.5 - 1.0 hours post-treatment, declining rapidly to about 0.5 mg/L at 24 hours post-treatment. Average total excretion of residues ranged from 76.5% at 8 hours to 103.1% at day 39, with an average for the four calves of 87.2%. Overall, 95% of excreted residues were eliminated in the first day of withdrawal, with urinary excretion being 1.2 to 2.5 times that of faecal excretion. At 8 hours, residues in kidney, liver, fat and muscle were 3.51, 1.29, 0.32 and 0.21 mg/kg, respectively, while residues in lung tissue were 0.92 mg/kg. Injection sites contained 1.5 to 3.9 mg/kg of ceftiofur residues.
At day 3, all samples contained less than 1.0 mg/kg of residues, with concentrations in muscle being 0.02 mg/kg. No residues were detectable in muscle at 21 and 39 days of withdrawal, but low concentrations (0.01 - 0.16 mg/kg) persisted in liver and kidney. Injection sites contained 0.01 - 0.40 mg/kg of residues at these withdrawal times. Residue depletion curves for the liver, kidney and lung were biphasic.

Further research was conducted in which 8 Holstein calves received 5 intramuscular injections at 24-hour intervals with ^{14}C-ceftiofur sodium (2.2 mg/kg BW), after which 2 calves were killed following each of 3, 20, 40 and 119 days withdrawal (Johnson *et al*, 1988). These data were combined with the 8-hour data from a related experiment using the same treatment regimen (Johnson *et al*, 1986) to demonstrate that depletion followed a biphasic curve in kidney, liver, muscle and lungs of the treated calves. Furthermore, they demonstrated a crossover at 20 days for the liver and kidney curves, so that while the kidney was identified as the target tissue up to 20 days withdrawal, the liver was recommended as the target tissue at longer withdrawal times. Values for k and $t_{1/2}$ calculated for these curves are given in Table 2.

Table 2. Residue decline rates in tissues calculated for calves treated with ^{14}C-ceftiofur sodium.

	Phase 1		Phase 2		
Tissue	k (days^{-1})	$t_{1/2}$(days)	k (days^{-1})	$t_{1/2}$(days)	Corr.
Kidney	0.42	1.66	0.022	31.5	0.999
Liver	0.41	1.69	0.012	57.8	0.931
Muscle[1]	0.60	1.16	0.008	86.6	0.836
Lung	0.55	1.26	0.013	53.3	0.988
Blood	1.24	0.56	0.033	21.0	0.995

[1] Calculation of muscle depletion rate for phase 2 compromised by analytical detection limit of 0.01 mg/kg.

Swine

The metabolic profile of ceftiofur in the urine and kidney of pigs treated with ^{14}C-ceftiofur sodium by intramuscular injection (Yein *et al*, 1990a) was compared with results obtained for oral administration of the drug to rats (Jaglan & Roof, 1990), demonstrating a match in the metabolite profiles in the kidneys of both the rats and the pigs (Jaglan *et al*, 1990a). In pig kidney, 62.6 ± 4.6% of the total residues were bound to macromolecules and the remainder was free residues, with DCD being the principal free metabolite. DFC is the major bound metabolite, as observed in other species. Residues of DFC were present in samples of fat (0.28 mg/kg), kidney (0.69 mg/kg), and liver (0.93 mg/kg) of 2 pigs slaughtered 24 hours after treatment and in the liver of one of two pigs slaughtered 48 hours post-treatment (0.36 mg/kg). The half-life for ceftiofur, measured as desfuroylceftiofur acetamide was calculated as 13.5 ± 2.61 hours after intramuscular injection and 12.2 ± 1.91 hours after intravenous dosing (Yein *et al*, 1990a).

Pharmacokinetic parameters for DFC in the blood of pigs treated with 3 mg CFAE/kg BW by intramuscular injection, on three successive days at 24-hour intervals, have been calculated and are given in Table 3 (Banting *et al*, 1991b). The table also lists these parameters which have been calculated for pigs receiving a single treatment of 3 mg CFAE/kg BW by intramuscular and by intravenous injection (Banting *et al*, 1991a). The reasons for the differences in calculated parameters for the single dose and 3-dose treatments are not clear, but may relate, in part, to differences in sampling times post-treatment used in the two studies.

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Table 3. Pharmacokinetic parameters of desfuroylceftiofur (DFC) in swine.

Variables	Swine[a]	Swine[b]	Swine[b]
Dose (mg/kg)	3 (times 3)	3 (times 1)	3 (times 1)
Route of Administ.	IM	IM	IV
Method of Assay	HPLC-DCA[c]	HPLC-DCA	HPLC-DCA
C_{max}(μg/mL)	12.46 ± 3.53	19.23 ± 7.88	19.62 ± 8.34
t_{max}(h)	2.33 ± 0.82	0.58 ± 0.20	1.11 ± 1.50
$AUC_{0\text{-}24h}$ (μg.h/mL)	136.63 ± 40.58	203.16 ± 121.06[d]	156.63 ± 58.32[d]
$t_{½}$(h)	15.74 ± 0.84	13.46 ± 2.61	12.24 ± 1.91
C_{min}, 24 h (μg/L)	2.62 ± 0.96	-[e]	-[e]

[a] Banting *et al*, 1991b, parameters calculated after last of 3 injections
[b] Banting *et al*, 1991a
[c] HPLC-DCA refers to total desfuroylceftiofur, assayed as desfuroylceftiofur acetamide
[d] $AUC_{0\text{-}\infty}$, μg.h/mL
[e] not calculated

The tissue and fluid distribution of DFC after administration of a single intramuscular injection of ceftiofur,

Table 4. Distribution of desfuroylceftiofur (DFC) in tissues (mg/kg) and fluids (μg/mL) of pigs treated with a single IM injection of 3 mg CFAE/kg BW.

Tissue	2.5 hr.	7.5 hr.	24 hr.
Plasma	13.64	8.31	3.25
Apical Lung	1.91	1.26	0.49
Diaphragmatic Lung	1.64	1.51	0.46
Bronchial Epithelium	1.65	1.49	0.62
Tracheal Epithelium	2.03	1.88	0.60
Tonsils	1.21	1.12	0.30
Kidney	4.33	3.02	1.06
Skin	1.73	1.17	0.57
Jejunum	1.22	1.00	0.41
Mesenteric Lymph Node	1.39	1.09	0.41
Synovial Fluid			
Right Stifle	1.07	0.46	0.29
Left Stifle	0.97	0.74	0.29
Cerebro-Spinal Fluid	<LOQ	<LOQ	<LOQ

Assay limits of quantification (LOQ): plasma, 0.14 mg/kg; kidney, 0.10 mg/kg; muscle, 0.03 mg/kg.

3 mg CFAE/kg BW, to swine has also been investigated for a 24-hour period following injection (Scantox & Cephac, 1994). This experiment indicated that, in healthy pigs, ceftiofur does not cross the blood brain barrier. Results of the residue analyses are presented in Table 4.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Lactating Cattle

Data were considered from studies in lactating cattle administered ^{14}C-ceftiofur by intramuscular administration which demonstrated the time from treatment to the appearance of peak concentrations of total residues in milk and also total residue depletion during and following last treatment (Jaglan *et al*, 1989a,b). In the pilot study (Jaglan *et al*, 1989b), a single cow (Table 5) was treated with 5 successive daily intramuscular doses of ^{14}C-ceftiofur sodium, 1 mg/kg BW (doses 1-3 at 21-hour intervals, doses 4 & 5 at 24-hour intervals).

Table 5. **^{14}C-ceftiofur residues in milk following 5 successive daily intramuscular injection of 1.1 mg/kg body weight of ceftiofur to a cow[a]**

Time Post-treatment (hrs.)	Total residues (μg/L[b]) ceftiofur equivalents)
3	15.3
6	34.1
9	38.0
12	43.6
15[c]	33.9
18[c]	20.4
21[c]	19.7
24[c]	18.3

[a] Based on samples from only one animal.

[b] Recalculated from mg/kg to μg/L using a specific gravity of 1.032 for milk.

[c] Includes one milking prior to these milking times.

Six Holstein cows (470-632 kg BW) were injected intramuscularly on five successive days at 24-hour intervals with 2.29 ± 0.02 mg ^{14}C-ceftiofur free acid-equivalents per kg BW (Jaglan et al, 1989b). Milk was collected at intervals of 12 and 24 hours following each treatment. Three screening tests which would detect the presence of microbiologically active residues in the milk samples (*Bacillus stearothermophilus* disc assay, or BSDA; *micrococcus luteus* cylinder plate assay, or C/P; Delvotest-P) were applied to samples listed in Table 6, with all tests yielding negative results. Actual concentrations of ^{14}C-ceftiofur equivalents present in the samples are shown in Table 6. LOD's claimed for the test kits were, respectively, BSDA, 0.08 - 0.1 mg/L; Delvotest-P, 0.05 mg/L; C/P, 0.015 mg/L.

Table 6. **^{14}C-ceftiofur equivalents (mg/L[a]) in milk collected from dairy cattle administered ceftiofur sodium via intramuscular injection on 5 consecutive days, at 24 hour intervals, at 2.3 mg/kg BW.[b]**

Time Post-treatment (Di + h)[d]	^{14}C Ceftiofur Equivalents (μg/L)[c] Total	Free[c]	Major Free Metabolite[c]
D1 + 12	79.9	28.2	6.9
D1 + 24	39.0		
D2 + 12	98.2	35.6	9.3
D2 + 24	45.1		
D3 + 12	105.4	36.0	9.3
D3 + 24	49.1		
D4 + 12	105.4	35.9	8.8
D4 + 24	51.3		
D5 + 12	111.8	37.1	9.8
D5 + 24	57.9	19.9	4.0
D5 + 36	31.5		
D5 + 48	19.4		
D5 + 60	10.1		
D5 + 72	12.9		
D5 + 84	10.7		
D5 + 96	11.4		
D5 + 108	10.9		
D5 + 120	9.2		

[a] Recalculated from mg/kg to μg/L using a specific gravity of 1.032 for milk.

[b] Mean value for milk collected from six cows.

[c] ^{14}C ceftiofur equivalents total residues present in milk as determined by total radioactivity (includes free metabolites that are not covalently bound to protein and the major free metabolite, desfuroylceftiofur cysteine disulfide, which is microbiologically active).

[d] (Di + h) refers to ith Dose (Di), where i = 1 to 5, and h hours after last treatment. For example, D1 + 12 refers to the sample taken at 12 hours after the first treatment.

Cattle

In a preliminary study (Johnson *et al*, 1985b), two calves and (124.5, 126.0 kg), two heifers (181.5, 181.8 kg) and a young bull (192.5 kg) were each given 5 successive treatments with 2.2 mg ^{14}C-ceftiofur sodium by intramuscular injection, with a 24-hour interval between injections. No significant differences in residues, as determined by total radioactivity (detection limit 0.02 mg/kg), were found in tissues collected from the calves and two of the heavier animals sacrificed after 8 hours withdrawal. Analysis of the normal edible tissues revealed that residues in kidney were highest (3.9 - 5.9 mg/kg), followed by liver (1.06-1.53 mg/kg), fat

(0.30-1.21 mg/kg) and muscle (0.16-0.30 mg/kg). Highest levels were found in the final (treatment 5) injection sites, ranging from 4.98-10.54 mg/kg, but were generally lower in the injection sites for the earlier treatments, although one injection site from treatment 4 contained 8.67 mg/kg. Injection sites from treatment 3 contained 2.0 mg/kg of residues, or less. In the remaining animal, sacrificed at 75 days after final injection with ceftiofur, traces of ceftiofur residues (<0.02 mg/kg) remained in three injection sites, while residues in the liver and kidney were 0.02 and 0.04 mg/kg, respectively.

The experiment was repeated using 6 calves (124.5-191.5 kg) which received the same dosage and treatment pattern, following which all six animals were sacrificed after 8 hours withdrawal (Johnson *et al*, 1986). Analysis by total radioactivity indicated the highest residue levels in normal tissues to be in kidney (5.54 ± 1.30 mg/kg), while the average residue found in muscle was 0.23 ± 0.05 mg/kg. Residues in injection sites were similar to those reported previously (Johnson *et al*, 1985b). Elimination of ceftiofur through urine and faecal clearance was rapid, with 80.5-90.2% of the dose eliminated after 8 hours and maximum concentrations were noted in the blood (5-10 μg/mL) 0.5 to 2 hours post-treatment, confirming earlier results (Johnson *et al*, 1985a).

A comparison of results obtained for the analysis of residues of ^{14}C-ceftiofur in kidney by total radioactivity and liquid chromatography demonstrated that much of the residue present does not include an intact cephalosporin nucleus (Jaglan & Gilbertson, 1991). Zero withdrawal kidney containing incurred residues of 7.2 mg/kg by total radioactivity contained 2.5 mg/kg ceftiofur equivalents, measured as DFC residues by HPLC. Residues were detectable in kidney after up to 3 days withdrawal for calves treated at 1.1 mg CFAE/kg BW/day on 5 successive days, but over 90% of the residues present did not contain an intact cephalosporin nucleus. Nonetheless, kidney is recommended as the target tissue.

Swine

Twelve swine (6 male, 6 female), 4-5 months old, weighing 35-46 kg, were treated by intramuscular injection on three successive days at 24 hour intervals with 5.108 ± 0.09 mg ^{14}C-ceftiofur free acid equivalents/kg BW and killed at 12 hours following the final injection (Yein *et al*, 1990a). Samples collected included the blood and urine, stomach contents at slaughter, liver, kidneys, lungs and tonsils, each as total organs, muscle from the right ham (about 100 g), an area 10 cm in diameter and 10 cm deep centred on the injection site, 200 g of abdominal fat and 100 g of skin with a small amount of adhering fat. The G.I. tract was washed clean and ground into a composite sample. Approximately 100 g of brain tissue and the mesentery gland (50 g) were also collected. All tissues were sub-sampled for analysis by liquid chromatography, with detection by both diode array and radioactive flow detectors.

Total ceftiofur concentrations in blood were in the range of 20 mg/L 2 hours after each injection, declining to about 10 mg/L after 12 hours and to 4-5 mg/L 24 hours after treatment. Analyses conducted on excreta revealed that 61.82 ± 4.70 % of the drug was eliminated in the urine and 10.75 ± 5.07 % was eliminated in the faeces prior to slaughter (collections at 24-hour intervals after treatments 1 and 2, 12 hours after treatment 3). Residues found in edible tissues collected at slaughter are given in Table 7. Residues found in the G.I. tract and gland tissues were similar to those found in liver, while lung tissue contained residues of 2.93 mg/kg total ceftiofur equivalents. Brain tissue contained 0.50 mg/kg total ceftiofur equivalents, the lowest residues found in the various tissues tested.

Table 7. **Total residues of ^{14}C-ceftiofur equivalents found in tissues of swine at slaughter (mg/kg).**

Tissue	Total Residues ^{14}C-ceftiofur, mg/kg[1]
Injection site	2.90 ± 1.28
Fat	1.49 ± 0.54
Kidney	4.47 ± 0.81
Liver	1.55 ± 0.18
Muscle	0.76 ± 0.24
Skin	1.22 ± 0.52

[1] Mean of determinations for twelve animals.

Other Residue Depletion Studies (with unlabelled drug)

Swine

A study was conducted in which 24 pigs (12 male, 12 female) were divided into four groups, each with 6 animals, and were dosed on three successive days at 24-hour intervals with 3 mg CFAE/kg BW by intramuscular injection (Banting *et al*, 1991b). The groups were sacrificed at 12 hours, 2 days, 5 days and 10 days, respectively, after the final injection. As shown in Table 8, analysis of tissue samples revealed that DFC residues were detectable in liver up to 2 days after the last injection. No detectable residues were found in tissue samples collected at 5 and 10 days post-treatment. Urine samples collected from 3 of the 6 animals slaughtered at 12 hours withdrawal were tested. One contained no detectable residues (LOD 0.1 mg/L), while the other two samples contained 4.29 and 1.87 mg/L DFC, respectively. Urine samples collected at days 2 (4/6), 5 (6/6) and 10 (6/6) contained no detectable residues of DFC.

Table 8. **DFC residues (mg/kg) in tissues collected from swine treated with three successive doses (24-hour interval) of CFAE at 3 mg/kg BW[a].**

Time from last injection	Fat	Injection Site	Liver	Kidney	Muscle
12 hours	0.40 ±0.08	0.19±0.11	1.51±0.72	2.17 ±1.02	N.D.
2 days	N.D.	N.D.	*	N.D.	N.D.

[a] Detection limit of LC method for DFC is 0.2 mg/kg in tissues.
N.D means not detected.
* Liver sampled from one animal contained 0.21 mg/kg DFC;
the other 5 livers contained no detectable residues.

Calves

Calves were treated with 1.1 mg CFAE/kg BW by intramuscular injection on 5 successive days (Jaglan & Cox, 1988). Kidneys collected at slaughter were analyzed and contained residues as follows (number of samples follows sampling time in brackets): 24 hours (6), 0.62 ± 0.12 mg/kg; 36 hours (7), 0.35 ± 0.07 mg/kg; 48 hours (7), 0.14 ± 0.04 mg/kg.

Twenty-five veal calves (39-68 kg), allocated into groups of five, each received 1 mg/kg BW ceftiofur by intramuscular injection on 5 successive days at 24-hour intervals (Banting *et al.*, 1989a). Blood was collected at intervals following the final injection of 0, 2, 4, 8, 16, 24, 48, 72 and 96 hours from 5 randomly selected calves. Groups of 5 calves each were slaughtered at intervals of 5, 10, 15 and 20 days after the final treatment and samples of injection site, muscle, liver, kidney, and fat were collected. Urine was collected from two calves 24 hours after the last treatment and also was collected from the bladder at slaughter, when possible. Samples were analyzed for DFC using a liquid chromatographic method of analysis with limits of quantitation as follows: plasma, 0.1 mg/L; urine, 1.0 mg/L; tissues, 0.2 mg/kg. Highest levels of DFC (2.3-5.0 mg/L) were found in the plasma samples taken at 2 and 4 hours, with concentrations declining rapidly to 0.39-0.74 mg/L at 24 hours, and to 0.00-0.17 mg/L at 48 hours. DFC was not detected at 72 and 96 hours following the final injection. DFC was present in one of two samples collected from each of two calves within 24 hours of treatment, but most urine samples collected at slaughter were negative. All tissue samples were negative at 5 and 10 days post-treatment. Fat and muscle samples were not analyzed for the 15- and 20-day withdrawal animals, but injection sites and livers contained no detectable residues. Kidney collected from one of 5 calves slaughtered after 15 days withdrawal contained 0.40 mg/kg DFC and kidney collected from one of 5 calves slaughtered at day 20 contained 0.27 mg/kg DFC, while the corresponding urine samples collected from these animals at slaughter contained 25.3 and 2.03 mg/L DFC, respectively.

Six healthy calves (54-73 kg) received a single intrsmuscular injection of ceftiofur sodium at a dosage of 1 mg CFAE/kg BW (Banting & Hewett, 1991). Three calves were killed after 2.5 hours withdrswal and the remaining three were killed at 7.5 hours after treatment. Results of the analyses of tissues collected from these animals are presented in Table 9.

Table 9. DFC residues in tissues from calves treated with a single dose of 1 mg CFAE/kg BW, mg/kg.

Tissue	t = 2.5 hrs.	t= 7.5 hrs.
Liver	1.42 ± 0.34	1.05 ± 0.53
Kidney	1.28 ± 0.07	1.20 ± 0.12
Injection site	1.86 ± 0.70	0.60 ± 0.29

Lactating Dairy Cattle

Forty-eight lactating Holstein dairy cows were assigned to six groups, with each animal receiving 2.2 mg CFAE/kg BW by intrsmuscular injection on 5 successive days (Robb *et al*, 1992). Milk was collected from the various groups at 1, 2, 4, 6, 8, 10, 12 or 14 hours after the first and last of the five daily injections. Results of HPLC analyses of the milk samples are presented in Table 10, while test kit results are discussed in the next section.

Table 10. DFC residues in milk collected at intervals as shown after the first and fifth injections, from cows treated with 2.2 mg CFAE/kg BW/day for 5 days, μg/L[a].

Collection Interval After Injection (hours)	Collected After First Injection		Collected After Fifth Injection	
	n	Mean	n	Mean
1	3	32.9	5	22.2
2	3	29.2	4	30.0
4	6	29.9	6	29.5
6	6	33.1	6	43.4
8	6	61.0	6	59.3
10	6	72.1	6	71.5
12	6	52.4	6	39.9
14	6	52.3	6	36.4

[a] Only values above the limit of detection (LOD) of 15 μg/L were included in the calculation of means; limit of quantification (LOQ) for the method is given as 50 μg/L.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Screening Tests for Milk

Milk collected from ten lactating cows which were treated with 1.1 mg CFAE/kg BW by intramuscular injection on five successive days was tested using the *Bacillus stearothermophilus* Disk Assay (BSDA), the Delvotest-P and the Charm Test II (Jaglan *et al*, 1988). Milk samples were collected at 12 and 24 hours after each injection and at 12-hour intervals to 144 hours after the final treatment. All samples tested negative by the BSDA, and the Delvotest-P. The Charm Test II detected residues in some samples taken at 12 hours after treatment.

The performance of the (BSDA), the Delvotest-P and the Charm Test II for the detection of ceftiofur in milk was determined using antibiotic-free control milk fortified with ceftiofur, using penicillin G as a positive control (Yein *et al*, 1990b). Under the conditions of the experiment, the detection limits for the three tests were established to be 50, 50 and 5 μg/L CFAE, for the BSDA, Delvotest-P and the Charm Test II, respectively. Most samples collected in a subsequent study with treated dairy cattle (Robb *et al*, 1992) gave a negative response to the Delvotest-P and BSDA, except for 1 cow whose milk tested positive at collection intervals of 2, 6, 10 and 14 hours after the first injection, for both assays. The Charm Test II gave negative results for all samples except those from one cow at the 2, 8 and 14 hour intervals and two cows at 10 hours. For milk collected following the fifth injection, samples from one cow were positive by the Delvotest-P at 6 and 14 hours, while the milk from one cow was positive at 6 hours by the BSDA. All other samples were negative by these tests. Milk samples from one cow were positive by the Charm II assay at 2, 6, 8 and 14 hours. Mean assay results for DFC residues in milk collected from the cows in this study have been reported in Table 10, and individual sample results are available which would permit comparison of test kit and HPLC assay results.

Thirty healthy lactating dairy cows in three herds of 10 animals each were given a daily dose of 1 mg CFAE/kg BW by intramuscular injection on 5 successive days (Jaglan *et al*, 1990b). Milk was collected pre-treatment and 12 and 24 hours after each dosing, plus at intervals of 12 hours up to 120 hours following the final injection. Samples were tested using the BSDA, Delvotest-P and the Charm Test II, plus by Cylinder Plate Assay (CPA) and an immunoassay, the Cite Probe. The microbial inhibition tests, the BSDA, Delvotest-P and the CPA, with detection limits of 50, 50 and 20 μg/L, respectively, gave negative results for 564 out of the total 568 samples collected. The remaining four samples were positive by all three tests. The Cite Probe, with a detection limit

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of 25 μg/L, gave 33 positive results out of 209 samples tested after the final dose, but the reliability of the results with this kit are questionable as it was used on previously thawed samples and after the expiry date of the kit. Most samples collected while the animals were on treatment were positive with he Charm Test II, which has a detection limit of 5 μg/L, with the rate of positives declining to 17/30 at 36 hours post-treatment. Urine samples collected during the treatment period and tested with the Live Animal Swab Test (LAST), a microbial growth inhibition test, were also mostly positive, but were generally negative after 36 hours withdrswal. However, there did appear to be a significant number of false positives with the LAST, so its reliability as a predictive test for clearance of ceftiofur residues was questioned.

Six lactsting dairy cows (mean weight 493 kg, mean milk production 23.2 kg/day) each received a single intramuscular injection of 2.2 mg ceftiofur active ingredient/kg BW to test the performance of an LC assay and of sixteen screening tests for β-lactam antibiotics for the detection of ceftiofur and its active metabolites (Belschner *et al*, 1994). Milk was collected pre-treatment and at approximately 14 and 24 hours after treatment, as composite samples from each cow for each sampling time. The average concentration of ceftiofur and metabolites detected by LC in the 14-hour samples was 0.030 mg/L (range 0.022-0.038 mg/L), while none of the 24-hour samples tested positive by the LC method (LOD 0.015 mg/L). The Idexx SNAP test kit (LOD 0.02 - 0.04 mg/L) gave positive results for 2 of 6 14-hour samples and 1 of 6 24-hour samples, while the Delvo-X-Press (LOD 0.004 - 0.02 mg/L) gave positive results for all 6 of the 14-hour samples and 1 of 6 24-hour samples. Estimated LOD's for the Idexx SNAP test and the Delvo-X-Press were based on the results of these experiments by comparison with HPLC results. The Charm Quantitstive test gave positive results for all milk samples at both 14 and 24 hours after treatment, but all other tests, including the Disk Assay (BSDA) gave negative results for all samples. It has been observed that problems with false positive test kit results may occur when tests designed for application to bulk milk samples are used on samples from individual cows (Cullor, 1992; Van Eenenaam *et al*, 1993).

Screening Tests for Tissue and Urine

An agar cylinder plate assay using M. luteus as the test organism was tested for the detection of ceftiofur residues in fortified samples of bovine muscle, fat, plasma and urine (Stahl, 1985). Recoveries of 75 - 106% were reported for the 20 ng/g and 20 μg/L concentration range in the tissue and fluid samples, respectively. A cylinder plate assay for ceftiofur in plasma using *Providentia alcalifaciens* as the test organism was subsequently validated to an LOD of 0.007 μg/mL and an LOQ of 0.02 μg/mL, with average recoveries for samples fortified at 0.025 and 0.30 μg/mL being 105 and 92%, respectively (Fate *et al*, 1993). Sequential freeze-thaw cycles of the samples resulted in decreases of 15% (1 cycle) and 35% (2 cycles).

Samples of kidney, liver, muscle and injection site were collected from 10 piglets (21-30 kg BW), treated at 24-hour intervals on 3 successive days with 5 mg CFAE/kg BW by intramuscular injection, which were slaughtered 24 hours following the last treatment. The samples were tested using the European Union Four Plate Test (FPT) and the New Dutch Kidney Test (NDKT), which have detection limits of 4 mg/kg for tissue, with the exception that the FPT will detect 1 mg/kg of ceftiofur residue in muscle tissue. All samples tested negative using these two tests (Guelen *et al*, 1989a). In a similar study with calves which received 1 mg CFAE/kg BW at 24-hour intervals on five successive days by intramuscular injection, no residues were detected at 22 hours or 5 days withdrawal using the FPT or the NDKT (Guelen *et al*, 1989b).

Twenty-four healthy pigs (99-117 kg) were divided into two groups, each consisting of 6 males and 6 females, with the two groups being treated, respectively, with 3 mg CFAE/kg BW/day and 5 mg CFAE/kg BW/day, by intramuscular injection for 3 successive days at 24-hour intervals (Yein *et al*, 1992a). The pigs were slaughtered at 11 - 12 hours after the final treatment and samples of liver and kidney were collected from each animal for analysis by the Swab Test on Premises (STOP) and the Calf Antibiotic and Sulfa Test (CAST). Kidney from one animal in each treatment group tested positive by the STOP and all livers tested negative. Using the CAST, the only positive test result was for one kidney from the 5 mg/kg BW treatment group. Kidney samples were also tested using the Four Plate Test (FPT), yielding positive results on plate 3, which uses *M. luteus* as the test organism, for 7 of the 12 samples from the 5 mg/kg BW treatment group (Yein *et al*, 1992b). No positive results were obtained on the other plates or for the animals treated at 3 mg/kg BW. Actual residues of CFAE were determined by HPLC analysis. Mean concentrstions for the kidneys from the two treatment groups were 3.35 ± 1.25 mg/kg (3 mg/kg BW) and 5.98 ± 2.21 mg/kg (5 mg/kg BW).

Six female calves (120-180 kg) each received five consecutive daily doses of 1.0 mg CFAE/kg BW by intramuscular injection and were slaughtered at 8 hours following the last injection (Stahl *et al*, 1992). Kidneys and final injection sites were collected and analyzed by the Four Plate Test (FPT) and by HPLC. While no microbiologically active residues were detected in the kidneys by the FPT, HPLC analysis revealed a mean concentration of 2.76 ± 0.40 mg/kg CFAE. One muscle injection site tested positive by the FPT, while the others tested negative. Injection sites were not assayed by HPLC. It was concluded that for the treatment regimen followed, slaughter following 8 hours withdrawal would not result in a large number of positive FPT results.

Chemical Methods

The stability of ceftiofur residues in milk has been investigated under various conditions of storage (Roof & Jaglan, 1991). Using control raw milk fortified at concentrations of 0.21 - 1.6 μg/mL and analyzed by HPLC, it was shown that residues are stable for 1 day at room temperature, 3 days under normal refrigeration and remained unchanged after 15 days storage at -20°C. A previous study with ^{14}C-ceftiofur residues demonstrated that residues of the ceftiofur metabolite DCD remained unchanged, as analyzed by HPLC, after 7 months storage at -20°C (Roof & Jaglan, 1989).

An HPLC method has been developed for the determination of DFC, the primary metabolite of ceftiofur, in cattle plasma which is based on release of bound residues using dithioerythritol in phosphate buffer (ph 8.7), followed by clean-up on C18 and SAX solid phase extraction cartridges (Jaglan *et al*, 1990c). The DFC is derivatized to form desfuroylceftiofur acetamide and analyzed on a C8 HPLC column under gradient conditions with UV detection at 254 nm. Recoveries were in the 90 - 100% range with a sensitivity of 0.1 mg/L. The methodology has also been applied to the determination of ceftiofur and desfuroylceftiofur related metabolites in bovine kidney, with a limit of quantification of 0.05 mg/kg and recoveries of 87 - 89 % from samples fortified at 0.11 - 5.35 mg/kg (Roof *et al*, 1994). The LOD was calculated to be 0.02 mg/kg for residues in bovine kidney samples.

In bovine muscle, the LOQ and LOD for the LC methodology were calculated to be 0.05 and 0.015 mg/kg, respectively, with recoveries in the 88 - 97% range for samples fortified in the range 0.1 - 10.0 mg/kg (Beconi-Barker *et al*, 1994a). The method has also been applied to bovine liver, with LOD and LOQ of 0.05 and 0.1 mg/kg, respectively, used as actual figures for the study (Beconi-Barker *et al*, 1994b). Recoveries obtained for samples fortified at 0.1, 1.0 and 10.0 mg/kg were 92.0, 87.5 and 93.5%, respectively. When applied to bovine fat, the same LOD and LOQ were determined as for bovine liver and recoveries were again similar (89.4 - 92.2%) in the 0.1 to 10.0 mg/kg fortification range (Beconi-Barker *et al*, 1994c). The methodology was tested for interferences from the cephalosporin antibiotics cefquinone, cefoperazone, cephapirin and cephacetril, all of which were removed by the derivatization and purification process in bovine muscle and kidney, except for cephapirin, which appears in the chromatogram at a retention time separated by about 1 minute from that of the derivatized DFC (Beconi-Barker *et al*, 1994d). Other antibiotics tested in the study , including neomycin, dihydrostreptomycin, spectinomycin, tetracycline and penicillin G, did not cause any interferences. No incurred tissues were tested in any of these validation studies for the HPLC method for tissues.

The HPLC method has been validated using fortified tissues for swine muscle and kidney. The LOQ was assigned as the lowest point on the calibration curve, which was 0.03 mg/kg for muscle and 0.1 mg/kg for kidney (Mignot *et al*, 1994). Recoveries reported were 88.0, 83.1, 79.6 and 74.7% for fortification levels of 0.1, 0.5, 2.0 and 10.0 mg/kg in kidneys, while in muscle the recoveries were 85.0, 80.9, 71.6 and 69.4%, respectively at fortification levels of 0.03, 0.1, 2.0 and 10.0 mg/kg.

A similar approach has been used to develop an HPLC assay for ceftiofur residues in milk (Roof & Jaglan, 1992; Hornish *et al*, 1994). The procedure employs basically the same steps as that described above for plasma, except that an SCX solid phase extraction cartridge is employed instead of the SAX cartridge. The LOD and LOQ given for DFC residues in milk are 15 and 50 μg/L, respectively, with a linear response from 0-560 μg/L and a correlation coefficient of 0.999 for the standard curve. DFC is identified as the marker residue for milk, corrected to total residue by multiplication by a factor of 1.3, based on recoveries from incurred samples containing ^{14}C-ceftiofur.

A method for the simultaneous determination of six β-lactam antibiotics, including ceftiofur, as residues in milk has recently been reported (Tyczkowska *et al*, 1994). In this method, the milk sample is diluted with acetonitrile, purified by ultrafiltration and separated on a phenyl HPLC column using ion-pairing reagents, following which parent compounds are quantitatively determined by their UV absorption at 210 (penicillin G, ampicillin, cloxacillin), 230 (amoxicillin) or 290 nm (cephapirin, procaine, ceftiofur). The eluent from the UV detector was directed into a splitter to reduce the flow rate from 300 µL/min to 4.3 µL/min prior to introduction into an electrospray source connected to a quadrupole mass spectrometer for the simultaneous determination of the compounds tested, except ceftiofur, for which no mass spectral data were reported.

A confirmatory procedure for total residues of DFC in milk has been developed which uses the derivatized DFC residues from the HPLC quantitative procedure for further analysis by thermospray HPLC/MS (Hornish *et al*, 1990). The method uses the pseudomolecular ion at m/z 487 (MH^+) and two fragment ions with m/z 326 and 243 for confirmation and was tested for quantitation based on monitoring of MH^+ from 0.1 to 0.8 mg/L, providing a linear correlation of 0.994, with an estimated limit of detection of 0.05 mg/L. The method requires as a minimum a quadrupole-based mass spectrometer with a suggested mass range of 2000 amu, with a thermospray interface and a low pulse HPLC pump suitable for use with a mass spectrometer. The method was successfully tested on incurred samples.

APPRAISAL

Ceftiofur is well-absorbed following intramuscular injection and has a relatively complex metabolism in mammals, rapidly forming both non-polar and polar metabolites, a significant proportion of which are bound to complex molecules in plasma and tissue. A number of the metabolites are biologically active, with desfuroylceftiofur being the primary metabolite found and the marker residue compound recommended for analysis. Excretion is primarily through the urine and elimination is rapid from both tissues and plasma, even with repeated doses, as demonstrated in a number of studies which were evaluated.

Studies conducted at therapeutic dose levels in swine and cattle indicate that residues are generally below 1 mg/kg in muscle within 12 hours after the last treatment, falling below detection limits in tissues within 2-3 days following the last treatment for most animals tested. Similarly, no residues were detected in milk samples taken at 24 to 48 hours following the last treatment. Depletion has been observed to follow a biphasic curve, with kidney being the target tissue for samples collected up to 20 days post-treatment. Beyond that date, liver may be the preferred target tissue, but residues detected will be negligible. There was no indication of significant persistence of residues at injection sites. Based on these considerations, kidney is recommended as the target tissue for national monitoring programs or investigations involving suspect animals. It is also recognized, however, that muscle is the primary tissue available for examination in international trade and thus may be the choice of tissue for monitoring of imported samples.

A number of bioassay screening tests are commercially available which should be suitable for the monitoring of milk, tissue, plasma and urine samples. For quantification, an HPLC method is available which converts desfuroylceftiofur related metabolites to desfuroylceftiofur acetamide for analysis. The detection limits for the methodology are in the range of 0.02 - 0.05 mg/kg for tissues and 15 µg/L in milk. A HPLC/MS confirmatory method has also been demonstrated for residues in milk.

Maximum Residue Limits

In reaching its decision on the MRLs for ceftiofur sodium, the Committee took into account the following:

- An ADI of 0-50 µg/kg of body weight was established based on a microbiological endpoint. This is equivalent to a maximum daily intake of 3000 µg for a 60-kg person;

- kidney and muscle were considered to be the appropriate target tissues;

- ceftiofur is used in lactating cows;

- a significant proportion of the residues is present as non-extractable protein bound material that does not include an intact β-lactam structure;

- desfuroylceftiofur is the appropriate marker residue and accounts for all residues that include an intact β-lactam structure; and

- limits of quantification for the HPLC method for desfuroylceftiofur are 50 μg/kg for kidney and muscle, 100 μg/kg for liver and fat and 50 μg/l for milk in cattle, and 30 and 100 μg/kg for pig muscle and kidney, respectively.

The Committee recommended the following MRLs for cattle and pigs, expressed as desfuroylceftiofur:

Muscle:	200 μg/kg
Liver:	2000 μg/kg
Kidney:	4000 μg/kg
Fat:	600 μg/kg
Milk (cattle):	100 μg/l

Based on these MRLs, the maximum theoretical daily intake would be 640 μg per person (Table 11).

Table 11. Theoretical maxium daily intake (TMDI) of residues of ceftiofur sodium (DFC)

Tissue	MRL as DFC (μg/kg)	Tissue Consumption (g)	TMDI (μg)
Muscle	200	300	60
Liver	2000	100	200
Kidney	4000	50	200
Fat	600	50	30
Milk	100 μg/L	1.5 L	150
Total			640

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Copyrighted Material

CHLORTETRACYCLINE

First draft prepared by
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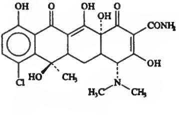
Pymble, Australia

IDENTITY

Chemical names:	7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Synonyms:	AC 13555; CL 13555; Chlortetracycline; W 4565; Aureomycin; Aureomycin 1000; AUROFAC; Duromycin; Emyrenil; Nidantin; Ossian; Ozoboi; Pietil; Prodoxol; Urinox; Uritrate; Uro-Alvar; Urotrate; Uroxin; Von Boch; Uroxol; Utibid.

Structural formula:



Chlortetracycline

Molecular formula:	$C_{22}H_{23}ClN_2O_8$
Molecular weight:	478.88

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:	Chlortetracycline, chlortetracycline hydrochloride or the calcium salt of chlortetracycline
Major Impurities:	Epichlortetracycline (8% max.)
Appearance:	A finely divided yellow powder
Melting point:	168-169°C
Optical rotation:	$[\alpha]_D^{23} = -275°$ (MeOH)

Solubility: Water - 8.6 g/L, MeOH - 17.4 g/L, EtOH - 1.7 g/L

Stability: Chlortetracycline hydrochloride and the calcium salt of chlortetracycline are stable 2 and 3 years, respectively, at room temperature.

Manufacture: Chlortetracycline is an antibacterial agent obtained by aerobic fermentation of a strain of *Streptomyces aureofaciens*. It is obtained commercially by large scale fermentation.

Mode of Action: All tetracyclines act by the inhibiting attachment of aminoacyl-t RNA to the A site on the 30S ribosome to prevent protein synthesis. Translation is inhibited by 1 molecule of the tetracycline per ribosome. It is postulated that a tetracycline-magnesium complex is formed at the ribosome, making it less flexible and therefore unable to bind aminoacyl-t RNA. It has been found that binding of tetracyclines to the 30S ribosome is dependent on proteins S7, S14 and S19 (Sande and Mandell, 1990).

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

The major agricultural use of the substance is in intensive poultry and swine husbandry and also in beef and cattle production, both as a therapeutic and prophylactic agent. Chlortetracycline is usually administered mixed in the feed at doses of 10-500 g/ton for poultry, sheep and swine or between 2 and 10 mg/kg bw per day for calves, cattle and sheep. Lower doses are generally prophylactic whereas higher doses are used therapeutically. Chlortetracycline is also used in combination with other drugs such as amprolium, clopidol, decoquinate, ethopabate, hygromycin B, monensin, penicillin, robenidine, roxarsone, sulfamethiazole and zoalene.

Chickens and Turkeys

Promote growth and improve feed efficiency; prevention and treatment of chronic respiratory disease; protection against, and treatment of infection by chlortetracycline sensitive organisms; control of sinovitis, prevention and control of coccidiosis; treatment of blue comb, infectious enteritis and sinusitis, mud fever and hexamitiasis.

Swine

Promote growth and improve feed efficiency; prevention and treatment of bacterical swine enteritis; maintenance of weight gain in the presence of atrophic rhinitis; reduce spread of leptospirosis; reduction of abortion rate.

Cattle

Promote growth and improve feed efficiency; prevention of foot rot; reduction of bacterial diarrhea and bacterial pneumonia; prevention of anaplasmosis, reduction of losses due to respiratory infections.

Sheep

Promote growth and improve feed efficiency; aid in reduction of losses due to enterotoxemia and vibronic abortion.

Horses

Promote growth and improve feed efficiency.

METABOLISM AND PHARMACOKINETICS

Metabolism

Chlortetracycline is moderately well absorbed from the intestinal tract and there is considerable evidence that it is not significantly metabolised. It is excreted from the animal body, in both feces and urine, either in the unchanged form or in a form with much reduced antimicrobial activity. Epichlortetracycline, to which the parent is readily converted, is the main identifiable 'metabolite' which is probably largely an artefact formed during isolation from biological material. In laboratory animals, the kidney and the liver are of major importance in the excretion of chlortetracycline.

Quantitative studies in which ^{14}C-labeled chlortetracycline hydrochloride was administered by oral, intraperitoneal (IP) and intravenous (IV) routes to adult male Wistar rats and by the IV route to male beagle dogs have been reported (Wulf and Eisner, 1961; Eisner and Wulf, 1962). Orally administered chlortetracycline was excreted primarily in the faeces, whereas after IP dosing the amounts recovered from feces and urine were approximately the same. Urinary excretion predominated after IV administration of chlortetracycline at 15 mg/kg but as the dose was raised, the ratio of urinary to fecal excretion shifted to favor fecal excretion at an IV dose of 60 mg/kg. Potential metabolites were identified by paper chromatography, preceded where necessary by separation using column chromatography. Quantification was based on radiometric analysis. By all routes of administration and excretion, the recovery of *in vitro* antimicrobial activity was significantly lower than the recovery of radioactivity. The major 'metabolite', was identified as 4-epichlortetracycline, which accounted for 23 to 35% of the radioactivity recovered in the urine in rat studies and 32 to 60% in the dog study. This epimer is essentially inactive in the microbiological assay. The extent to which epimerisation occurs *in vivo*, if at all, is not clear, since this epimerisation is known to occur readily chemically and it is possible that epimerisation occurred under the conditions used for extraction of fecal samples. Indeed, in an *in vitro* experiment, approximately 20% of ^{14}C-labeled chlortetracycline added to normal dog urine was recovered as the 4-epichlortetracycline after 24 hours. Very small amounts of isochlortetracycline in the urine and feces of some of the animals were also detected. This compound is readily formed when chlortetracycline is subjected to a range of conditions, from neutral to alkaline. When ^{14}C-labeled or unlabeled 4-epichlortetracycline was administered IV to rats, there was little or no conversion of this epimer to a microbiologically active material (chlortetracycline). The authors concluded that "like tetracycline and demethylchlortetracycline, chlortetracycline appears not to be metabolized to any significant degree by rats or dogs in spite of the finding of 4-epichlortetracycline and isochlortetracycline in the excreta of these animals" (Wulf and Eisner, 1961).

Pharmacokinetics in Laboratory Animals

Oral doses of chlortetracycline ranging from 6 to 800 mg/kg bw were administered to female white rats and to guinea pigs (Eisner et al, 1953). In general, there was no direct proportional correlation of serum chlortetracycline concentration with dose. Administration of the same dose daily for nine days gave higher serum levels than those found with the single doses. Simultaneous administration of various organic acids, such as citric acid, increased serum chlortetracycline leves in doses ranging from 6 to 800 mg/kg.

Rats given single oral doses of 75 mg/kg bw of chlortetracycline attained plasma levels averaging 2.1 mg/L one hour post dosing. Plasma concentrations declined to 0.8 mg/L by six hours after dosing (Berte and Vandoni, 1962)

Beagle dogs given single oral chlortetracycline doses of 25 mg/kg bw showed peak serum levels ranging from 0.40 to 1.9 mg/L two hours after dosing which declined to an average of 0.21 mg/L after 24 h (Kandis, 1958). When the dogs were given a single IV dose of 10 mg/kg chlortetracycline, serum levels averaged 6.6 mg/L at one hour post dosing; declining to 2.4 mg/L at 8 h, 0.29 mg/L at 24 h and 0.06 mg/L at 48 h.

Adult white rabbits were given single oral doses of two different formulations of chlortetracycline at 20 mg/kg by stomach tube (Neuschl, 1991). Serum levels of chlortetracycline averaged 2.3 mg/L three hours after dosing; declining to 0.82 mg/L after 12 h and 0.09 mg/L after 24 h.

Pharmacokinetics in Turkeys

Single doses of 10, 15, and 20 mg/kg bw of chlortetracycline hydrochloride in aqueous solution were administered orally by gavage directly into the crops of small Beltsville White turkeys following a 17-hour fasting period (Pollet et al, 1984). The resulting plasma levels of chlortetracycline suggest that plasma concentrations were linearly related to dose. Peak plasma levels were reached 2 to 6 hours post-dosing, with the peak time being latest for the largest dose.

Investigations in several species have shown that divalent cations, such as calcium, have a suppressant effect on the absorption of tetracycline antibiotics. The co-administration of organic acids such as citric acid with the tetracycline antibiotic enhances blood serum levels of the tetracycline, probably because such acids form complexes with and sequester divalent cations thereby negating their depressant effect. Additional single oral dose studies in turkeys confirmed that the presence of calcium and magnesium in the birds' drinking water produced significantly lower blood chlortetracycline levels than were achieved when citric acid was added to the dosing solution. When minerals present in the gastrointestinal tract were kept to a minimum, the addition of citric acid to the dosing solution had little effect on the resulting plasma chlortetracycline levels (Pollet et al, 1984).

A study in which turkeys were fed a diet containing 440 mg/kg of chlortetracycline with and without 1.5% sodium sulfate after an overnight fast failed to show any effect of sodium sulfate on chlortetracycline levels in blood taken three hours after initiation of the medicated feed (Tindall and Berger, 1966a).

A study in turkeys where chlortetracycline was administered intravenously at a dose of 0.9 mg/kg b.w. demonstrated that chlortetracycline was rapidly removed from the blood and excreted via the bile (Dyer, 1988). The ratio of the peak bile/plasma concentrations was 254:1 at 2 h and was greater than 1:1 between 10 and 240 min. Over the period 4 hours after dosing, about 8.5% of the administered dose was accounted for in the bile.

Pharmacokinetics in Chickens

The oral time-course data for chickens dosed with chlortetracycline at 25 mg/kg followed by determination of chlortetracycline serum levels over a 24-hour period fitted a 2-compartment model adequately. Addition of citric acid to the oral chlortetracycline dose made little difference to the absorption rate constant obtained for chlortetracycline alone. The uptake of orally administered chlortetracycline was rapid but only a small fraction was absorbed. Co-administration of citric acid not only increased the mean serum concentration significantly but also produced distinctly higher elimination and distribution rates for chlortetracycline. After 4 h, the serum levels of birds dosed with either chlortetracycline alone or a chlortetracycline-citric acid combination were approximately the same (Pollet et al., 1983).

In another study, broiler chickens given chlortetracycline in water at 400, 800 and 100 mg/gallon, either alone or co-administered with citric acid, gave blood levels of chlortetracycline which failed to show any consistent correlation with the presence or absence of citric acid in the dose (Berger, 1982c). The effect of aflatoxin on the chlortetracycline blood levels of broiler chicks administed in combination has also been studied. There was a significant decrease in half-life levels of chlortetracycline and a significant increase in the systemic clearance in birds receiving IV chlortetracycline together with 2.5 mg/kg aflatoxin in the feed over control birds receiving chlortetracycline alone (Miller and Wyatt, 1985).

Two different drinking water formulations on chlortetracycline given to broiler chickens by oral gavage (10.8 mg/kg bw) gave chlortetracycline blood level averaging 0.13 mg/mL at 2 h and 0.20 mg/mL at 6 h. After 24 h the blood chlortetracycline levels in the majority of birds was below 0.015 mg/L, the limit of detection for the assay method (Schumacher, 1968).

In another study, chlortetracycline was administered via the drinking water to chickens at 120 mg/L for 7 days. The chlortetracycline blood concentrations, shown in Table 1, peaked at 0.14 mg/L 8 h after start of treatment and declined somewhat thereafter. The average daily intake of chlortetracycline ranged from 17.4 to 23.7 mg/kg bw over the 7 days of the treatment.

Table 1. Average Chlortetracycline Intake and Blood Levels in Chickens Given 120 mg/L of Chlortetracycline in Drinking Water for 7 Days (Gingher, 1989b).

Hours of Medication	Bird Average Weight (kg)	Chlortetracycline Intake (mg/kg)	Blood Chlortetracycline (mg/L)
0	1.218		ND
2	NM	NM	0.067 (0.049-0.107)
4	NM	NM	0.064 (0.049-0.074)
8	NM	NM	0.140 (0.086-0.225)
24	1.413	23.7	0.067 (0.051-0.085)
72	1.462	18.5	0.084 (0.055-0.131)
120	1.642	19.4	0.090 (0.044-0.129)
168	1.734	17.4	0.043 (0.024-0.067)

NM = Not Measured; ND = Not detected

Two studies were conducted in which different formulations of chlortetracycline were administered via the drinking water at a concentration of 264 mg/L (Berger, 1971; Schumacher, 1968). The resulting blood levels of chlortetracycline are shown in Table 2. It is interesting to note that, while the younger (smaller) birds consumed somewhat more chlortetracycline on a bw basis, the resulting blood concentrations were lower. Chlortetracycline levels in the blood of most birds were below the limit of detection of the assay by 24 hours after withdrawal of medicated water.

Table 2. Average Blood Chlortetracycline (CTC) Levels (in mg/L) in Chickens Given 264 mg Chlortetracycline/L of Drinking Water for 3 Days.

Reference	Berger, (1971)			Schumacher, (1968)	
Average Body Weight, kg	1.77	1.82	2.09	0.95	0.93
Av. Daily CTC Intake, mg/kg	31.9	28.8	25.1	36.8	36.6
Time on Medication	Average Blood Chlortetracycline Levels (mg/L)				
2-3 h	0.32	ND-0.33	0.23	ND-0.03	ND-0.08
6 h		ND-0.23	ND-0.23	0.11	ND-0.18
24 h	0.4	0.24	0.14	0.15	0.2
72 h	0.4	0.27	0.17	NM	NM
Withdrawal Time					
24 h	ND-0.03	ND-0.02	NM	NM	NM
48 h	ND-0.02	ND	ND	NM	NM
72 h	ND-0.02	ND-0.012	ND	NM	NM

ND = Not Detected; NM = Not Measured

Several studies have been conducted where chlortetracycline was given in the feed at 300 mg/kg for seven days (Gingher, 1989e, 1990b, 1990f). The resulting levels of chlortetracycline in the blood, presented in Table 3, show considerable differences in blood chlortetracycline concentrations between studies. The differences between data is probably due to differences in calcium levels in the basal diets. In these studies, blood chlortetracycline levels were inversely proportional to the level of calcium in the basal diets. This is not unexpected as many investigators have shown that calcium will depress absorption of chlortetracycline. The studies also show that medication of chickens via the feed is a reliable method of providing therapeutic levels of chlortetracycline which are well maintained throughout the period of administration of medicated feed.

Table 3. **Average Blood Chlortetracycline (CTC) Levels (mg/L) in Chickens Fed 300 mg/kg CTC in the Diet for 7 Days.**

Reference	Gingher (1989e)	Gingher (1990b)	Gingher (1990b)	Gingher (1990f)
Calcium in feed %	1.28	0.88	0.88	0.79
Average Body Weight, kg	1.52	1.03	1.06	0.88
Av. CTC Daily Intake, mg/kg	28.9	34.7	33.2	35.7
Hours medication	Blood Chlortetracycline (mg/L)			
0	ND	ND	ND	ND
2	0.081	0.118	0.136	0.411
4	0.108	0.184	0.203	0.413
6	NM	0.173	0.246	0.444
8	0.218	0.216	0.234	0.504
24	0.105	0.157	0.191	0.411
72	0.112	0.148	0.123	0.317
120	0.086	0.112	0.122	0.283
168	0.056	0.118	0.112	0.256

ND = Not Detected; NM = Not Measured

Accumulation of Residues in Eggs

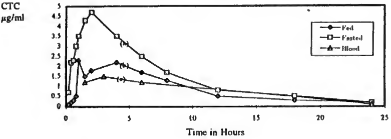
In an early study, Broquist and Kohler (1954) found no residues of chlortetracycline in eggs from hens fed 220 mg/kg chlortetracycline in the feed. When the level in feed was raised to 2200 mg/kg, eggs had chlortetracycline residues ranging from 0.15 to 3.1 mg/kg in whole egg.

Meredith et al (1965) dosed laying hens with 1000 mg/kg chlortetracycline in the diet, showed residues averaging 0.05 mg/kg of whole egg. Cooking by poaching or scrambling reduced the antibiotic activity significantly, but failed to destroy all residual antibiotic activity.

Pharmacokinetics in Swine

Chlortetracycline hydrochloride was administered intra-arterially (11 mg/kg) and as an oral drench (33 mg/kg) to ten 21.0 to 31.5-kg pigs. Five of the pigs were fasted 18 hours prior to dosing and five of the pigs were fed *ad libitum* prior to dosing. The average plasma chlortetracycline concentration data for the orally dosed pigs are shown in Figure 1 (a,b). The mean volume of distribution, determined by area-under-the-curve calculations, for the fasted pigs (0.967 ± 0.210 L/kg) was significantly less ($P<0.05$) than the mean volume of distribution for the fed pigs (1.39 ± 0.31 L/kg). Mean total body clearance of the drug was also significantly less ($P<0.05$) in the fasted pigs (0.165 ± 0.055 L/kg/h) as compared with the fed pigs (0.307 ± 0.053 L/kg/h). The elimination constants were not found to be statistically different [($P<0.05$): 0.1811 L/kg ± 0.0057 for the fasted pigs; 0.2260 ± 0.0461 for the fed pigs]. The bioavailability for both groups was similar; 19.12% $\pm$ 8.3% for the fasted pigs and 17.88% $\pm$ 5.3% for the fed pig (Kilroy et al, 1990).

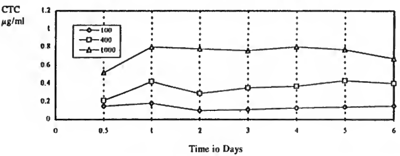
Figure 1. Chlortetracycline (CTC) Plasma Concentrations Following Single Oral Dose of 33 mg/kg Body Weight by Oral Gavage to Fed and Fasted Pigs



Ten pigs, weighing 22.2 to 25.0 kg and not fasted prior to treatment, were orally-gavage-dosed with two different soluble powder formulations of chlortetracycline. Blood chlortetracycline concentrations for the first 24 hours following dosing are shown in Figure 1(c). These data suggest that peak blood levels were achieved somewhat later than was seen in the work Kilroy et al (1990). Exept for one sample from the 6-h bleeding, which was much higher than any of the other 6-h samples, these results agree well with the results of Kilroy et al for nonfasted pigs (Berger, 1967b).

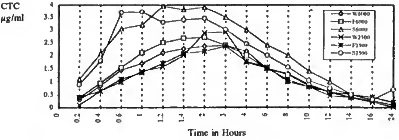
Three groups of six pigs which weighed 34.5 to 44.1 kg were fed corn-soy diets *ad libitum* which were fortified with chlortetracycline HCl at 100, 400 or 1000 mg/kg. Chlortetracycline coocentrations were determined in plasma samples collected over a 6-day period. Plasma chlortetracycline levels reached a plateau withio 24 hours after initial access to the feed (Figure 2) and were proportional to the dose of drug consumed (r^2=0 97) (Kilroy et al, 1990).

Figure 2. Chlortetracycline Concentrations in Plasma of Pigs Fed Corn-Soy Diets *ad libitum*, Fortified with CTC HCl at 100, 400 or 1000 mg/kg.



The influence of feed preparation on the pharmacokinetics of orally administered chlortetracycline was demonstrated by Sutter and Wanner (1990). Pigs were divided into 3 groups which received a dry, a moist, or a very wet, soupy diet each containing chlortetracycline concentrations of 6000 and 2500 mg/kg, respectively. The results shown in Figure 3, demonstrate a significantly higher chlortetracycline bioavailability in animals fed the very wet diet compared with the moist or dry diets although the amount fed furnished a dose of 40 mg chlortetracycline/kg bw in each case.

Figure 3. CTC Serum Concentrations after Oral Dose of 40 mg/kg of Body Weight. W = Dry, F = Moist and S = Very Wet

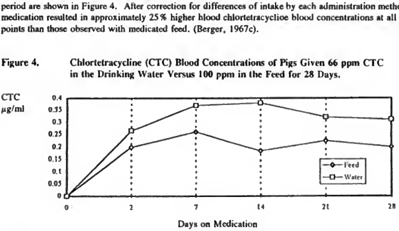


A study conducted at South Dakota State University indicated that metallic cations have an effect on chlortetracycline absorption (Wahlstrom et al, 1982). Calcium sulfate was shown to be a satisfactory calcium source, but it did not influence chlortetracycline absorption. This study also showed that dietary levels of sodium sulfate up to 2.13% increased serum chlortetracycline levels. Increase in dietary phosphate levels also had the same effect.

Blood chlortetracycline concentrations of pigs given access to drinking water containing 66 mg/kg chlortetracycline with an equal concentration of sulfamethazine over an eight day period reached 0.135 mg/L of blood after one day, and remained at about that level for the remaining eight-days. Blood chlortetracycline levels dropped to values around the LOD of the assay method (0.015 mg/L) by 24 hours after withdrawal of the drug (Wang, 1971b).

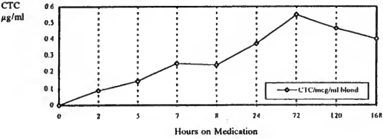
Blood chlortetracycline concentrations in pigs given chlortetracycline in feed or in drinking water for a 28-day period are shown in Figure 4. After correction for differences of intake by each administration method, water medication resulted in approximately 25% higher blood chlortetracycline blood concentrations at all sampling points than those observed with medicated feed. (Berger, 1967c).

Figure 4. Chlortetracycline (CTC) Blood Concentrations of Pigs Given 66 ppm CTC in the Drinking Water Versus 100 ppm in the Feed for 28 Days.



Blood chlortetracycline concentrations from pigs given free choice access to drinking water containing 120 mg/kg chlortetracycline for 7 days is shown in Figure 5. Average blood chlortetracycline levels rose rapidly for the first 6 hours, and then more slowly until day 3. Individual variations in blood concentrations make it doubtful if any real differences can be expected after the first day on medication. Average concentration of chlortetracycline in lung tissue on day 7 was 0.264 mg/kg. Average daily intake of chlortetracycline was 14.96 mg/kg bw (Berger, 1989d).

Figure 5. Chlortetracycline (CTC) Blood Concentrations of Pigs Given 120 ppm CTC in Drinking Water for 7 Days.



In another study pigs were given drinking water containing 147 mg/kg chlortetracycline for seven consecutive days. Blood and lung concentrations of chlortetracycline were measured after 1, 3, and 7 days on the treatment. Concentrations of chlortetracycline in the lung tissue averaged 60 to 85% higher than those found in the blood (Rooney, 1990).

There were no significant differences in chlortetracycline levels in blood of pigs receiving 110 mg/kg chlortetracycline in the feed for 28 days, supplied by different formulations (Berger, 1984, Garces, 1981). Within each study the blood concentrations averaged in a range of 0.11-0.43 mg/L over the dosing period.

The average values of data from studies in which pigs received 165 mg/kg chlortetracycline in feed for 7 consecutive days showed that blood concentrations of chlortetracycline rose steadily for the first 24 hours on medicated feed, after which average levels declined slightly by 7 days. The premixes used to prepare these feeds also contained sulfamethazine, and in three cases, penicillin (Rooney, 1989c). Pigs receiving 300 mg/kg chlortetracycline in feed for 7 consecutive days (Berger, 1989c; Berger, 1990; Gingher, 1990a) gave blood levels of the drug in two of the three comparisons which were very similar to those obtained with the 165 mg/kg results above (Gingher, 1990a).

Pharmacokinetics in Cattle

The plasma and tissue concentration and pharmacokinetics of chlortetracycline were determined in milk-fed and conventionally fed Holstein calves by Bradley et al (1982). The pharmacokinetics of chlortetracycline after a single intravenous dose of 11 mg chlortetracycline/kg bw were similar for both milk-fed and conventionally-fed calves (Figure 6). The drug was rapidly distributed from plasma into the peripheral compartment but was slowly eliminated, with detectable levels of chlortetracycline continuing for 72 hours after dosing. Milk-fed calves had a larger area under the plasma level curve following a single oral dose of 22 mg chlortetracycline/kg bw. Futhermore, a larger fraction of the dose was absorbed together with a smaller volume of distribution and a smaller overall body clearance rate. The mean plasma concentrations are shown in Figure 7. The chlortetracycline concentration in tissues following an oral dose was greatest in the kidney, followed by liver, heart, skeletal muscle, spleen and brain. Tissue depletion closely paralleled the decline in plasma concentration. These investigators suggest that the effects seen in milk-fed calves would probably be enhanced by administration of the drug in the milk replacer, as practiced in veal calf management. A study by Luthman and Jacobsson (1983), showed that when calves were given a dose of 50 mg chlortetracycline/kg bw in water, milk replacer or milk, the serum concentrations of chlortetracycline were lower when administered in milk replacer or milk (Figure 8). However, the serum chlortetracycline concentrations obtained by all three modes of administration can be considered quite satisfactory.

Figure 6

Mean Plasma Concentrations of CTC in Calves after Intravenous Administration of a Single Dose of 11 mg CTC/kg Body Weight

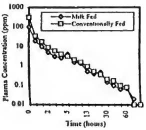


Figure 7

Mean Plasma Concentrations of CTC in Calves after Oral Administration of a Single Dose of 22 mg CTC/kg Body Weight

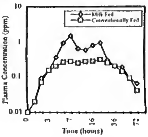
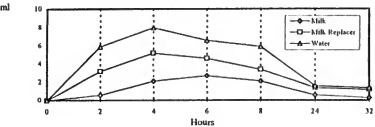


Figure 8. Serum Concentrations of Chlortetracycline in Calves after Oral Administration of 50 mg/kg of Body Weight in Water, Milk Replacer and Cows Milk.



A more recent study measured blood concentrations of chlortetracycline following administration of 700 mg chlortetracycline in a milk replacer to young Holstein calves twice daily over a 7-day period (Rooney, 1988a). On the first day of treatment average chlortetracycline blood levels peaked at 0.76 mg/L four hours after the first feeding of medicated milk replacer. On days 1, 3, 5, and 7 of treatment, average blood levels from samples taken four hours after the morning feeding were between 0.88 and 1.01 mg/L. Lung samples collected at euthanasia, four hours after the last feeding of medicated milk replacer were 1.21 to 1.90 mg/kg of tissue.

Similar data has been obtained following administration for seven consecutive days of either a soluble powder formulation of chlortetracycline in milk replacer at the dose of 13.3 mg/kg bw administered once daily (Rooney, 1989a) or with one soluble bolus of chlortetracycline twice daily, providing approximately 22 mg chlortetracycline/kg bw per day (Goodale, 1988). Blood chlortetracycline concentrations averaged 1.48 mg/L eight hours after the initial feeding of medicated milk replacer. On days 2, 3, 6 and 7 of treatment blood chlortetracycline levels from samples taken four hours after medication averaged between 1.47 and 1.61 mg /L. Chlortetracycline levels of lung samples collected at euthanasia, 4 hours after the last medicated feeding, were 2.05 to 2.93 mg/kg of tissue. On the first day of the bolus treatment, average blood chlortetracycline levels peaked at 0.204 mg/mL eight hours after the first treatment. Average blood levels of chlortetracycline from samples taken on days 3, 5, and 7 of treatment were between 0.71 and 0.92 mg/L. Lung samples collected at euthanasia contained a mean of 2.12 mg/kg.

In a much earlier study with somewhat older calves medicated once daily by bolus at approximately 5.5 mg/kg bw, blood chlortetracycline levels ranged from 0.13 to 0.69 mg/L during a three day treatment period (Shor, 1961b). Blood chlortetracycline levels gradually declined to an average of 0.06 mg/L four days after the last treatment.

Experiments conducted in young calves, compared the depletion of chlortetracycline from the blood following cessation of treatment with various oral dosing forms of chlortetracycline at a total daily dose of 22 mg/kg bw (Berger, 1974; Berger and Garces, 1981). The results of these studies, summarized in Table 4, show good agreement within each study regarding the magnitude and depletion of blood chlortetracycline for the various formulations. However, much higher blood chlortetracycline levels were seen in one study than the other, yet the bolus formulation tested was exactly the same for both studies. The calves showing the higher blood chlortetracycline levels were younger and received the medication divided between 2 daily doses, while the calves exhibiting the lower blood chlortetracycline levels received the medication once daily. The younger calves were fed a milk replacer diet only, while the older calves received a calf starter grain mix in addition to the milk replacer diet. It is probable that these older calves had experienced some rumen development, which according to the work of Bradley et al. (1982) would be expected to cause a lower absorption of chlortetracycline and a more rapid elimination of the drug from the body. This hypothesis is supportes by data shown in Table 4.

Table 4. Depletion of Blood Chlortetracycline Levels in Calves Following Cessation of Treatment with Soluble Boluses, Soluble Powder Formulations or Tablets for 5 or 10 Days at 22 mg Chlortetracycline/kg Body Weight Daily

Reference	Berger, 1974	Berger, 1974	Berger, 1974	Berger and	Berger and
Formulation[1]	B	SP	SP	B	T
Administered	Once Daily	Once Daily	Once Daily	Twice Daily	Twice Daily
Average Weight, kg	80.4	86.7	87.2	48.4	47.4
Days on CTC	10	10	10	5	5
Withdrawal Time	Chlortetracycline, mg/kg of tissue				
1 hour	0.41	0.72	0.42	1.83	1.93
2 hours	0.4	1.32	0.47	1.79	2.06
4 hours	0.42	1.22	0.57	2.03	1.97
8 hours	0.62	0.64	0.73	2.48	2.15
12 hours	0.58	0.48	0.68	NM	NM
1 day	0.33	0.19	0.35	1.42	1.46
2 days	0.14	0.07	0.13	0.72	0.82
3 days	0.07	0.04	0.07	0.42	0.55
4 days	0.04	0.03	0.04	0.32	0.39
5 days	0.02	0.02	0.03	0.15	0.23

[1] Formulation: B = soluble bolus; SP = soluble powder; T = tablet; NM = Not Measured

Studies have also been conducted in which older calves averaging 172-193 kg were given 22 or 11 mg/kg bw chlortetracycline in the feed for 4 or 5 days. The animals receiving the 22 mg/kg dose showed peak chlortetracycline levels of 0.454 and 0.458 mg/L at 12 and 16 h post feeding which declined to an average of 0.346 mg/L by 24 h after feeding (Craig, 1992). Lung tissue obtained 12 hours after the fifth daily medication contained an average of 1.166 mg/kg chlortetracycline. Cattle receiving 11 mg/kg daily had peak chlortetracycline blood levels of about 0.210 mg/L 12 and 16 hours after consuming the medicated feed which declined to an average of 0.148 mg/L by 24 hours post dosing (Biroc, 1992b). These levels agree very well with the earlier work of Alford (1970) who found that cattle receiving 10 to 12 mg chlortetracycline/kg bw in the feed for 3 days averaged blood levels of 0.235 mg chlortetracycline/L on the second and third days of receiving the medicated rations.

Tissue Distribution

Tissue distribution data for chlortetracycline is predominantly confined to experiments in laboratory animals with less evidence available from food animals.

In a study of the intestinal absorption in rats, tissue levels of chlortetracycline at 1 to 5 hours after dosing are shown in Table 5. Tissue levels were highest in liver and kidney at all times (Berte and Vandoni, 1962). In a study in which adult white rabbits were given single oral doses of two different formulations of chlortetracycline by stomach tube at 20 mg/kg, levels of the drug twenty-four hours after dosing were highest in liver, averaging 1.84 mg/kg, followed by kidney, lung and heart. No measurable chlortetracycline was found in muscle tissue in this experiment (Neuschl, 1991). Studies in rats (Buyske et al, 1960), mice and rabbits (Miller and Wyatt, 1985) have shown that chlortetracycline has a great affinity for bones and teeth, and remains in these tissues and structures for long periods of time.

Table 5. Chlortetracycline Levels in Tissues and Plasma of Rats Following a Single Oral Dose of 75 mg/kg bw (Berte and Vandoni, 1962).

	Chlortetracycline (mg/kg)				
Time After Dosing, min	Plasma	Lungs	Brain	Liver	Kidney
60	2.1±0.56	5.2±0.13	0.11±0.04	16.2±0.6	21.8±6.4
120	1.1±0.2	3.8±2.1	0.09±0.03	21.4±0.9	20.1±4.9
180	0.8±0.4	2.3±1.0	0.02±0.01	15.2±1.2	14.8±3.2
240	0.7±0.6	2.2±0.9	0.03±0.02	10.0±0.7	11.2±1.0
360	0.8±0.3	2.1±0.45	0.03±0.01	5.3±1.0	8.7±0.66

^{14}C-labeled chlortetracycline was administered IV to two beagle dogs to study the distribution throughout the body. Four hours after dosing the liver contained most radioactivity, followed in decreasing order by the kidney, ileum, jejunum, heart and duodenum. A large proportion of the recovered radioactivity was found in the urine, intestinal contents and bile. With the exception of subcutaneous fat, radioactivity was found throughout all tissues and fluids examined (Kelly, 1964). A summary of the findings is presented in Table 6.

Table 6. Total Micrograms of Chlortetracycline in the Whole Tissues of Dogs Four Hours After an Intravenous Dose of 10 mg/kg (Kelly, 1964).

Tissue	Dog A	Dog B	Tissue	Dog A	Dog B
Liver	5638	7782	Jejunum	559	723
Kidney	805	718	Ileum	755	790
Heart	634	529	Cecum	168	40
Lungs	366	426	Colon	255	156
Brain	69	60	Rectum	101	154
Bile	2750	3240	Stomach Contents	39	31
Spleen	123	102	Duodenal Contents	146	238
Pancreas	173	164	Jejunal Contents	845	876
Uterus	50	80	Ileal Contents	2146	2128
Trachea	36	44	Colon Contents	2481	1293
Esophagus	70	92	Rectal Contents	21	749
Stomach	230	347	Diaphragm	150	181
Duodenum	538	540	Urine	20711	N.M.

Dog A Body Weight 6.2 kg and Dog B 8.0 kg; NM = Not Measured

TISSUE RESIDUE DEPLETION STUDIES

Turkeys

Chlortetracycline residues in turkeys were measured in body fluids and tissues at 2, 8 and 24 hours following a single oral dose of of 15 mg/kg bw (Table 7). This study clearly shows that chlortetracycline levels are highest in the kidneys and/or liver at all three times, suggesting that either kidney or liver should be considered the target tissue (Pollet et al, 1984). These findings are supported by a study in which turkeys were given chlortetracycline bisulfate via the drinking water at the rate equivalent to 264 mg/kg of chlortetracycline HCl for 14 days (Berger, 1972). The results show conclusively that concentrations of chlortetracycline are highest and most persistent in the kidney, followed by the liver (Table 8), as would be expected since these are the two organs directly involved in the excretion of chlortetracycline.

Table 7. Concentration of Chlortetracycline in Tissues and Plasma Samples Following Oral Administration of 15 mg Chlortetracycline/kg Body Weight to Turkeys (Pollet et al, 1984)

	Hours After Chlortetracycline Administration		
Tissue	2	8	24
	Chlortetracycline, mg/kg		
Liver	3.05 ± 2.01	2.32 ± 1.37	0.61 ± 0.44
Kidney	8.76 ± 7.24	10.84 ± 8.19	2.57 ± 0.35
Red Muscle	0.23 ± 0.16	0.28 ± 0.17	0.05 ± 0.04
White Muscle	0.09 ± 0.06	0.15 ± 0.15	0.05 ± 0.02
Brain	0	0.5 ± 0.01	0.07 ± 0.05
Plasma	0.6 ± 0.44	0.44 ± 0.25	0.11 ± 0.05

A number of tissue residue experiments have been conducted where therapeutic doses of chlortetracycline were administered to turkeys via the drinking water or feed. A summary of the residue depletion data from kidney and liver tissues of turkeys medicated via the drinking water or feed are presented in Table 9.

Table 8. **Summary of Chlortetracycline Residues in Tissues of Turkeys Following Medication with 264 mg/kg Chlortetracycline in Drinking Water for 14 Days (Berger, 1972)**

	Chlortetracycline, mg/kg of Tissue			
Withdrawal Day	Muscle	Liver	Kidney	Skin/Fat
0	0.27	1.04	3.82	0.22
1	ND - 0.04	0.15	0.56	0.16
2	ND - 0.03	0.08	0.34	0.12
3	ND - 0.04	0.17	0.46	0.12
4	ND	ND - 0.08	0.3	0.08
5	ND	0.07	0.26	0.05

ND = Not Detected

Table 9. **Concentrations of Chlortetracycline in Liver and Kidney Tissues of Turkeys Receiving Chlortetracycline in the Drinking Water**

Reference	Berger, (1972)	Berger, (1982b)	Shor, (1962)	Shor, (1962)	Shor and Gale (1965)	Gutzman, (1990c)
Conc. in Water, (mg/kg)	264	106	528	528	528	528
Days on Medication	14	14	3	3	14	14
Chlortetracycline Average Daily Intake, (mg/kg)	17.4	16	63.3	71.7	19.8	26.4
Average Bird weight, (kg)	4.3	1.85	1.06	1.06	6.95	6.81
Withdrawal Day	Chlortetracycline, mg/kg of Liver					
0	1.04	0.26	1.00	1.56	0.36	0.38
1	0.15	0.06	0.12	0.14	ND-0.04	ND-0.22
2	0.08	0.04	0.10	0.10	ND-0.04	ND-0.22
3	0.17	ND	NM	NM	NM	NM
4	ND-0.08	NM	ND-0.05	ND-0.06	NM	NM
5	0.07	NM	NM	NM	ND-0.03	ND
Withdrawal Day	Chlortetracycline, mg/kg of Kidney					
0	3.82	1.24	3.73	11.52	1.57	2.20
1	0.56	0.24	0.80	0.91	0.13	0.89
2	0.34	0.17	0.60	0.73	0.12	0.13
3	0.46	ND	NM	NM	NM	NM
4	0.30	NM	0.26	0.40	NM	NM
5	0.26	NM	NM	NM	ND-0.08	0.09

NM = Not Measured; ND = Not Detected

Chickens

In common with other species, residues of chlortetracycline are the greatest and persist the longest in liver and kidney tissues of chickens. This is demonstrated in Table 10, with data from chickens which received 110 mg/kg of chlortetracycline in feed for 51 days followed by a 5-day feeding of a diet containing 550 mg/kg chlortetracycline (Gingher, 1979). At zero-day withdrawal from medicated feed, residues of chlortetracycline averaged 3.9 mg/kg in kidney, 0.79 mg/kg in liver, 0.27 mg/kg in muscle, less than 0.04 mg/kg in fat and less than 0.06 mg/kg in skin. By day 4, chlortetracycline could not be detected in muscle, fat and skin, while liver averaged less than 0.05 mg/kg and kidney averaged less than 0.25 mg/kg. It can be concluded that when chlortetracycline is not detectable in liver and kidney, residues will also be absent from all other edible tissues. Therefore, depletion of chlortetracycline residues in these two marker tissues has been emphasised in this review.

Table 10. **Residue Depletion in Tissues from Chickens which Received 110 mg/kg Chlortetracycline in Diet for 51 Days Followed by 550 mg/kg in the Diet for 5 Additional Days (Gingher, 1979).**

		Chlortetracycline mg/kg of tissue				
Withdrawal Day		**Muscle**	**Liver**	**Kidney**	**Fat**	**Skin**
0	Average	0.27	0.79	3.9	ND-0.04	ND-0.06
	Range	0.18-0.36	0.54-1.08	2.65-7.10	ND-0.09	ND-0.10
3	Average	ND-0.026	ND-0.06	0.38	ND	ND-0.12
	Range	ND-0.028	ND-0.08	0.31-0.47	----	ND-0.12
4	Average	ND	ND-0.05	0.25	ND	ND
	Range	----	ND-0.05	0.20-0.32	----	----

ND = Not Detected

A summary of residue data from chickens treated via the drinking water at levels of 120 to 528 mg/kg for periods ranging from 3 to 14 days is shown in Table 11 (Berger, 1982a; Gingher, 1989a; Stoner, 1983). As would be expected, the magnitude of the residues are in direct proportion to the concentration of chlortetracycline in the drinking water. Liver is essentially free of chlortetracycline residues two days after withdrawal, while measurable amounts of chlortetracycline (0.04 to 0.15 mg/kg) persist in kidney four days after withdrawal.

Four studies were conducted in which broiler chicks received 220 mg/kg chlortetracycline in the diet for the first three weeks of life. In each experiment the basal ration contained approximately 0.8% calcium and 1.5% of sodium sulfate to provide conditions for maximum absorption of the chlortetracycline. The average residue depletion data from liver and kidney tissues are shown in Table 12. Residues averaged from 1.45 to 2.18 mg/kg in kidney and 0.42 to 0.75 mg/kg in liver at zero-day withdrawal. Residues were below 0.1 mg/kg in liver by 3 to 5-days withdrawal. Residues in kidneys ranged from 0.05 to 0.11 mg/kg chlortetracycline at the 7-day withdrawal point (Berger, 1967e; Tindall and Berger, 1966b,c,d).

Table 11. Residues in Liver and Kidney Tissues From Chickens Receiving Chlortetracycline in the Drinking Water

Reference	Gingher, 1989a		Berger, 1982a		Stoner, 1983	
Chlortetracycline in Water, mg/kg	120		264		528	
Days on Medication	7		14		3	
	Chlortetracycline, mg/kg					
Withdrawal Day	Liver	Kidney	Liver	Kidney	Liver	Kidney
0	0.276	NM	0.36	>2.87	0.87	3.78
1	ND-0.049	NM	ND-0.08	0.37	0.07	0.36
2	ND-0.03	NM	ND-0.06	0.32	ND-0.03	0.23
3	ND	NM	NM	NM	NM	NM
4	ND	NM	NM	NM	ND	0.09

ND = Not Detected; NM = Not Measured

Table 12. Residues of Chlortetracycline in Liver and Kidney Tissues from Chickens Receiving 220 mg/kg Chlortetracycline in Feed for First Three Weeks

Reference	Berger,1967e		Tindall and Berger, 1966b		Tindall and Berger, 1966c		Tindall and Berger, 1966d	
Ca in diet, %	0.8		0.81		0.81		0.81	
Na_2SO_4 in diet %	1.5		1.5		1.5		1.5	
Average bw, kg$^{(1)}$	0.379		0.372		0.413		0.4	
Average Daily CTC Intake, mg/kg$^{(2)}$	22		24.2		19.8		22.4	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	0.53	2.01	0.42	1.45	0.71	1.91	0.75	2.18
3	NM	NM	NM	NM	0.04	0.27	NM	NM
4	NM	NM	NM	NM	0.04	0.24	NM	NM
5	ND-0.04	0.21	ND	0.17	ND-0.05	0.24	ND-0.03	0.15
7	ND	0.09	ND	0.08	NM	NM	ND	0.11

[1] Average weight at end of medication period; [2] Average daily chlortetracycline intake last week of medication; NM = Not Measured; ND = Not Detected

Two separate studies (Drain, 1962a ; Gingher, 1980) in older chickens administered 220 mg/kg chlortetracycline in the feed showed residue levels below 0.05 and 0.5 mg/kg in liver and kidney, respectively, 3 days after withdrawal of medication. Results are shown in Table 13.

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Table 13. **Residues of Chlortetracycline in Liver and Kidney Tissues from Older Chickens Receiving 220 mg/kg Chlortetracycline in Feed**

Reference	Drain, 1962a		Gingher, 1980	
Calcium in diet, %	0.47		0.83	
Average Body Weight, kg	approx. 1.8		2.23	
Days on Medication	5		55	
Withdrawal Day	Chlortetracycline, mg/kg of Tissue			
	Liver	Kidney	Liver	Kidney
0	0.66	0.42	0.71	0.75
1	ND-0.06	0.25	NM	NM
3	ND	0.07	ND-0.03	0.3
4	NM	NM	ND-0.04	0.28
6	ND	0.05	NM	NM

NM = Not Measured; ND = Not Detected

Residue depletion data from a study in which 10-week old broiler chickens received diets containing 0.4% calcium and levels of chlortetracycline ranging from 880 to 2200 mg/kg for a 5-day treatment period is presented in Table 14 (Drain, 1962b). Chlortetracycline levels found were related but not directly proportional to the quantity of the drug consumed. Residues in the liver were all at or below 0.1 mg/kg after 1 day withdrawal of medicated feed. Kidney tissues were all below 1 mg/kg chlortetracycline after one-day withdrawal, but measurable residues (0.04 to 0.40 mg/kg) were still found in kidney tissue 10 days after withdrawal.

Table 14. **Residues in Liver and Kidney Tissues From 10-week old Broiler Chickens which Received High Levels of Chlortetracycline in the Diet for 5 Consecutive Days (Drain, 1962b)[1]**

	Chlortetracycline; mg/kg Liver (L) and Kidney (K) Tissue							
Dose	880 mg/kg		1320 mg/kg		1760 mg/kg		2200 mg/kg	
WT, days	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	0.9	6.44	1.3	6.93	1.27	10.44	1.75	11.82
1	ND-0.02	0.16	0.04	0.25	0.07	0.46	0.07	0.49
3	ND-0.035	0.12	ND-0.04	0.31	0.04	0.24	ND-0.03	0.2
6	ND	0.08	ND-0.02	0.09	ND-0.025	0.17	ND	0.11
8	ND	0.07	ND-0.025	0.18	ND-0.03	0.11	ND-0.03	0.17
10	ND-0.02	ND-0.05	ND	0.1	ND-0.03	0.19	ND-0.03	0.15

[1] Diet contained 0.4% Calcium; ND = Not Detected

The results of two more recent studies conducted with 300 mg/kg chlortetracycline in feed for a 7-day treatment period are shown in Table 15 (Gingher, 1988b, Gingher, 1990f). Liver tissues contained 0.228 to 1.10 mg/kg of chlortetracycline at the zero-day withdrawal point, while kidney tissues contained 2.45 to 3.05 mg/kg chlortetracycline. Residues in liver were all below 0.4 mg/kg at one day withdrawal. Very small amounts of chlortetracycline were still apparent in some kidney samples after 20 days withdrawal.

Table 15. **Residues of Chlortetracycline (mg/kg) in Liver and Kidney Tissues of Chickens Receiving 330 mg/kg Chlortetracycline in Feed for 7 Days**

Reference	Gingher, 1988b	Gingher, 1990f	
Calcium, % in diet	1.28	0.79	
Average Body Weight	1.479	1.04	
Average Daily Intake, CTC, mg/kg	28.6	31	
Withdrawal Day	Liver	Liver	Kidney
0	0.328	0.99	2.72
1	ND-0.031	NM	NM
2	ND-0.026	NM	NM
3	ND	NM	NM
6	NM	ND-0.027	0.211
8	NM	NM	0.144
10	NM	NM	0.091
13	NM	NM	0.079
15	NM	NM	0.053
17	NM	NM	0.041
20	NM	NM	ND-0.038

ND = Not Detected (<0.025 mg/kg); NM = Not Measured

Chicken Eggs

Two recent studies have been conducted in which chlortetracycline was administered to White Leghorn laying hens at 300 mg/kg in feed (Gingher, 1989c) and at 120 mg/kg in the drinking water (Gingher, 1989d). In both studies the treatment periods were for seven consecutive days. Eggs were collected during and following the treatment period. In the feed study, average daily chlortetracycline intakes ranged from 27.7 to 47.4 mg chlortetracycline/bird and averaged 35.7 mg chlortetracycline/bird/day during the 7-day medication period. Residues ranging from 0.037 to 0.044 mg/kg of whole egg were found in 4 of 10 eggs assayed at the zero-day withdrawal. At one day withdrawal, residues ranging from 0.035 to 0.042 mg/kg of whole egg were found in 3 of 10 eggs assayed. No chlortetracycline residues were found in the eggs at subsequent withdrawal days. In the water medication study, average daily chlortetracycline intake ranged from 21.8 to 36.3 mg chlortetracycline/bird and averaged 26.9 mg chlortetracycline/bird/day over the 7-day treatment period. Residues ranging from 0.040 to 0.043 were found in 4 of 10 eggs assayed at zero day withdrawal. At one-day, withdrawal residues of 0.040 and 0.042 mg/kg chlortetracycline were found in 2 of 10 eggs assayed. No chlortetracycline residues were found in the eggs at subsequent withdrawal days. In these studies, the whole egg was assayed and the limit of detection of the method was 0.0375 mg/kg chlortetracycline of whole egg.

Katz et al. (1972) studied continuous feeding at levels of chlortetracycline of 55, 110, 165 and 220 mg/kg in the feed. Egg production and chlortetracycline residues in eggs were studied over a 4 month period. Their data showed that 11.1, 83.8, 94.8 and 100% of eggs had measurable residues of chlortetracycline from hens fed 55, 110, 165 and 220 mg/kg in the feed, respectively. The assay procedure used was more sensitive than that used by the Gingher (1989), measuring chlortetracycline concentrations as low as 0.021 mg/kg. Measurable residues at the 165 and 220 mg/kg feed levels were no longer detectable 3 days after removal of the medication.

Roudaut et al. (1989), treated laying hens with feed containing 600 mg/kg chlortetracycline for a seven day period. Residues were measured separately in the yolk and albumin fractions of the egg. A summary of the residue data obtained in this study is presented in Table 16. Measurable residues were found in the albumin for 5 days and in the yolk for 9 days after withdrawal of the medication from the feed. The LOD of the method was 0.01 mg/kg for albumin and 0.06 mg/kg for yolk. About 70%, of the chlortetracycline excreted via the egg, was found in the yolk and about 30% in the albumin.

Table 16. Chlortetracycline Residues in Albumin and Yolk Fractions of the Egg and the Whole Eggs Following a 7 Day Treatment with 600 mg/kg in the Feed (Roudaut et al., 1989)

	Chlortetracycline, mg/kg (Mean ± SD)		
Days of Treatment	**Albumin**	**Yolk**	**Whole Egg**
7	0.1 ± 0.03	0.41 ± 0.11	0.19 ± 0.05
Withdrawal Day			
1	0.07 ± 0.02	0.48 ± 0.12	0.19 ± 0.05
2	0.03 ± 0.009	0.50 ± 0.15	0.17 ± 0.05
3	0.02 ± 0.004	0.37 ± 0.10	0.12 ± 0.03
4	0.02 ± 0.003	0.3 ± 0.08	0.1 ± 0.02
5	0.01 ± 0.003	0.23 ± 0.07	0.08 ± 0.02
6	<0.01	0.14 ± 0.03	0.05 ± 0.01
7	NM	0.11 ± 0.02	0.04 ± 0.008
8	NM	0.08 ± 0.02	0.03 ± 0.007
9	NM	0.06 ± 0.01	0.02 ± 0.004
10	NM	<0.06	

NM = Not Measured

Swine

Residue depletion data for edible tissues of pigs fed 440 mg/kg chlortetracycline in feed for 14 days is presented in Table 17. These data demonstrate that swine are similar to other species in that the highest and most persistent residues occur in kidney and liver tissue (Berger, 1983). Korsrud and MacNeil (1987), using an HPLC procedure for measuring chlortetracycline in tissues, have concluded that the kidney should be used as the target tissue for the detection of chlortetracycline residues in swine. Therefore, as for the other species, major emphasis in this summary have been placed on the depletion of residues from liver and kidney tissues.

Table 17. Chlortetracycline Residue Depletion in Tissues from Pigs Which Received 440 mg/kg Chlortetracycline in Feed for 14 Days (Berger, 1983).

	Chlortetracycline, mg/kg of Tissue			
Withdrawal Day	**Muscle**	**Liver**	**Kidney**	**Fat**
0	0.75	1.88	>3.78	0.2
1	0.28	0.65	1.69	0.06
2	0.23	0.68	1.5	0.06
3	0.14	0.53	0.8	0.04

Residue depletion data for pigs which received 66 to 198 mg/kg chlortetracycline in the drinking water for 5 to 28 consecutive days is summarized is Table 18 (Berger, 1968, 1989e; Drain, 1961a). When 66 mg/kg chlortetracycline was given for 28 consecutive days, residues in both liver and kidney were higher and more persistent than when the same dose was given for only 5 days. As would be expected, the greatest residues were obtained for the pigs receiving the highest dose of chlortetracycline in the water. Over 90 percent of the chlortetracycline was removed from the liver and kidney during the first 24 hours after withdrawal of the medicated water. The rate of depletion was much lower in subsequent days.

Table 18. **Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Receiving CTC 66 to 198 mg/kg in Drinking Water.**

Reference	Berger, 1968		Berger, 1968		Berger, 1989e		Drain, 1961a	
Chlortetracycline, mg/kg in water	66		66		120		198	
Days on medication	28		5		7		5	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	2.04	2.05	1.13	2.05	0.93	2.05	2.72	2.05
1	NM	NM	NM	NM	NM	NM	0.12	0.43
2	NM	NM	NM	NM	NM	NM	0.05	0.31
4-5	0.07	0.16	NM	NM	NM	NM	0.12	0.26
7	0.05	0.14	ND-0.03	0.06	NM	NM	0.06	0.17
10	0.07	0.12	ND-0.04	ND-0.04	ND-0.036	0.43	NM	
14-15	ND-0.04	0.07	ND	ND-0.03	ND	0.042	ND-0.04	NM
20-21	0.03	0.09	ND	ND-0.025	ND-0.034	ND-0.036	ND-0.03	0.08
28-30	ND	ND-0.03	ND	ND	ND	ND-0.029	ND	0.06

NM = Not Measured; ND = Not Detected

Data from studies in which pigs received 110 or 330 mg/kg chlortetracycline in feed for periods ranging from 31 to 98 consecutive days are summarised in Table 19. Although, in most cases, the premixes furnishing the chlortetracycline also contained sulfamethazine and penicillin or nitrovin, only chlortetracycline residue data appears here. When 110 mg/kg chlortetracycline was fed for 31 days and for 98 days, the concentration of chlortetracycline in liver and kidney was over twice as high for the 31-day feeding compared to that for the 98-day feeding period, and depletion time was correspondingly longer. This is, in part, probably due to the size of the pigs at the time of drug withdrawal. The average weight at drug withdrawal time was 33.6 kg for the 31-day feeding period as compared to 83.8 kg for the 98-day feeding period. The younger, smaller pigs consumed more feed than the older pigs, therefore receiving a higher chlortetracycline dosage on a bw basis. When 330 mg/kg chlortetracycline was fed for a 98-day period, residue levels of chlortetracycline were about twice those for pigs fed 110 mg/kg chlortetracycline for the same time (Alford, 1967; Berger, 1966b; Sass and Messersmith, 1964; Stoner, 1962b).

In recent studies when 165 mg/kg chlortetracycline in feed was given for 7 consecutive days, average chlortetracycline concentration was 0.95 mg/kg in liver and 1.38 mg/kg in kidney at 0-day withdrawal (Table 20). By the 10-day withdrawal, all residues in both liver and kidney were below 0.05 mg/kg of tissue (Gingher, 1990e; Rooney, 1989d).

Table 19. **Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Which Received 110 or 330 mg/kg Chlortetracycline in Feed**

Reference	Stoner, 1962b		Sass and Messersmith, 1964		Berger, 1966b		Alford,1967	
Days on Medication	31		98		98		63	
Average Weight, kg	33.6		83.8		89.9		49.5	
Drug in feed, mg/kg	110		110		330		110	
	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
Withdrawal Day	**(L)**	**(K)**	**(L)**	**(K)**	**(L)**	**(K)**	**(L)**	**(K)**
0	0.85	1.01	0.35	0.39	0.71	0.83	0.74	1.02
3	0.09	0.15	NM	NM	NM	NM	NM	NM
5	0.08	0.15	ND-0.04	0.06	0.11	0.16	NM	NM
7	0.08	0.14	0.04	0.1	0.13	0.14	ND-0.08	0.12
10	NM	NM	ND-0.04	0.04	0.07	0.09	NM	NM

NM = Not Measured; ND = Not Detected

Table 20. **Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Which Received 165 mg/kg Chlortetracycline in Feed for 7 Days**

Reference	Gingher, 1990e		Rooney, 1989d	
Tissue	**Liver**	**Kidney**	**Liver**	**Kidney**
Withdrawal Day	**Chlortetracycline, mg/kg of Tissue**			
0	0.943	1.38	0.96	1.38
10	ND-0.033	0.041	NM	NM
12	ND-0.027	ND-0.033	NM	NM
15	ND	0.031	ND	ND-0.037
20	ND	ND-0.025	ND	ND-0.030
25	NM	NM	ND	ND

NM = Not Measured; ND = Not Detected (<0.025 mg/kg)

Additional studies have been conducted in which 300 and 400 mg/kg chlortetracycline in feed were given to pigs for 7 consecutive days (Berger, 1989a ; Gingher, 1990d). Chlortetracycline residues averaged 1.45 mg/kg in liver and 1.93 mg/kg in kidney at zero-day withdrawal of the 300 mg/kg of chlortetracycline. For the pigs receiving the 400 mg/kg chlortetracycline dose in feed, zero-day withdrawal residue levels averaged 1.32 and 2.69 mg/kg for liver and kidney, respectively. Residue levels drop to less than 10% of these values by three days after withdrawal of the medicated feed, but the depletion of the residues is more gradual thereafter, for these tissues (Table 21). Both the liver and the kidneys are directly involved in the removal of chlortetracycline from the body via the bile and the urine.

Table 21. **Chlortetracycline Residues Depletion in Liver and Kidney Tissues of Pigs which Received 300 to 400 mg/kg Chlortetracycline in Feed for 7 Days**

Reference	Berger, 1989a		Ginger, 1990d		Gingher, 1990d	
Chlotetracycline, mg/kg in Feed	300		300		400	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)					
	(L)	(K)	(L)	(K)	(L)	(K)
0	1.67	1.57	1.23	2.29	1.32	2.69
3	NM	NM	0.129	0.121	0.111	0.148
5	NM	NM	0.102	0.087	0.083	0.107
7	NM	NM	0.069	0.08	0.067	0.069
10	NM	NM	0.058	0.06	0.034	0.047
12	NM	NM	ND-0.067	0.041	ND-0.049	0.047
15	0.044	0.051	0.036	0.038	0.046	0.048
20	ND	ND-0.029	ND-0.034	ND-0.035	ND-0.037	0.035
25	ND	ND	NM	NM	NM	NM
30	ND	ND	NM	NM	NM	NM

NM = Not Measured; ND = Not Detected (<0.025 mg/kg)

Cattle

The depletion of chlortetracycline from edible tissues of calves following a 10-day treatment at a dose of 22 mg/kg bw daily is presented in Table 22 (DeLay, 1973). These were young calves, averaging 42 kg bw, receiving a milk replacer diet with medication supplied by soluble boluses once daily. Residues at zero-day withdrawal were highest in kidney, followed by liver, muscle and fat. After ten days withdrawal, residues of 0.06 to 0.15 mg/kg and 0.14 to 0.16 mg/kg remained in liver and kidney tissue, respectively. As has been shown in other species, the kidney and liver can be considered the target tissues.

A summary of recent chlortetracycline depletion studies from liver and kidney of young calves following therapeutic doses of the drug from various dosage formulations for 7 consecutive days is shown in Table 23. The calves in two of the studies received a diet of whole milk (Berger, 1989b; Goodale, 1988c), while in the other two studies the calves received a diet of reconstituted milk replacer (Rooney, 1988b; 1989b). The daily doses of chlortetracycline ranged from 13.3 to 30.2 mg/kg bw. Residues of chlortetracycline at zero-day withdrawal were not directly proportional to the administered dose. The comparative results at day zero withdrawal between bolus and powder formulations, where the average daily dose of 21.7 mg/kg via the bolus formulation exceeded the average of 13.3 mg/kg given in the soluble powder formulation, are particularly intriguing. The soluble powder gave liver and kidney residue values of 13.7 and 19.2 mg/kg, well in excess of those from the bolus formulation, 1.82 and 2.18 mg/kg in liver and kidney, respectively. No ready explanation could be advanced for such disparate results. Residues from liver and kidney samples did not exceed 0.05 mg/kg after the 25-day withdrawal or the 45-day withdrawal, respectively. However, detectable residues were still found in kidney samples at the last withdrawal point for each of the studies. Although not shown in Table 23, no detectable chlortetracycline residues were found in fat samples after the zero-day withdrawal, while residues were not detected in muscle samples by withdrawal day 25 to 35, depending on the study. An earlier study in which young calves were given chlortetracycline soluble boluses at the rate of 22 mg/kg body weight per day, residues averaged 4.57 mg/kg in kidney, 3.22 mg/kg in liver, 1.26 mg/kg in muscle, and 0.49 mg/kg in fat at zero-day withdrawal. At 10 days withdrawal, chlortetracycline residues in kidney samples ranged from 0.14 to 0.16 mg/kg and in liver samples from 0.06

to 0.10 mg/kg (DeLay, 1973).

Table 22. **Depletion of Chlortetracycline Levels from Tissues of Calves Following Oral Treatment at 22 mg/kg bw Daily for 10 Days (DeLay, 1973)**

Withdrawal Day		Chlortetracycline, mg/kg of tissue			
		Muscle	Liver	Kidney	Fat
0	Average	1.26	3.22	4.57	0.49
	Range	1.08-1.55	2.70-3.65	4.30-4.90	0.31-0.63
3	Average	0.47	1.39	1.26	0.15
	Range	0.38-0.59	1.11-1.80	1.00-1.55	0.10-0.20
7	Average	0.14	0.27	0.45	0.04
	Range	0.07-0.21	0.12-0.46	0.24-0.70	0.03-0.06
10	Average	0.03	0.09	0.15	Neg-0.03
	Range	0.02-0.04	0.06-0.10	0.14-0.16	Neg-0.04

Neg = Below the LOD of the assay

Residue depletion data from an earlier study are available for calves following a 5-day medication with chlortetracycline soluble powder given by oral drench (Berger, 1967d). These calves averaged 67 kg body weight and were receiving alfalfa hay and a calf fitting ration ad libitum. So, rumen development was probably well advanced. Average residues of 1.44 and 2.18 mg/kg chlortetracycline were seen in liver and kidney, respectively, at zero-day withdrawal. After 3 days withdrawal, liver samples contained no detectable chlortetracycline and kidney samples averaged 0.05 mg/kg.

Results of three studies in which cattle were dosed daily with chlortetracycline via the feed at average rates of 22, 11 and 4.4 mg/kg body weight are summarized in Table 24. In the first study, cattle averaging 208 kg bw were given 22 mg/kg bw of chlortetracycline for 14 consecutive days (Gingher, 1981). Residues were greater than 5.0 mg/kg in kidney and averaged 1.9 mg/kg in liver at zero-day withdrawal. No residues were detected in liver by day 10, while kidney decreased to 0.05 mg/kg at this time. Kidney samples continued to average 0-0.05 mg/kg at 14 and 21 days withdrawal. In the second study, two-year old cattle received 11 mg chlortetracycline/kg bw daily for 61 consecutive days (Berger, 1965). Zero-day residue concentrations in this study were roughly one-half of those seen in the first in which twice the daily dose of chlortetracycline was fed. By ten days withdrawal, residues were not detected in liver while kidney samples averaged 0.05 mg/kg chlortetracycline. In the third experiment, cattle were fed 4.4 mg chlortetracycline/kg bw daily, together with an equal amount of sulfamethazine, for 28 days (Munger, 1978). Chlortetracycline residues averaged 1.37 and 0.52 mg/kg in kidney and liver, respectively, at the zero withdrawal sampling. No detectable residues of chlortetracycline were found in any tissues at the 10-day or subsequent withdrawals (Table 24).

Table 23. **Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Calves Following Various Oral Dosing Forms for 7 Days.**

Reference	Berger, 1989b	Goodale, 1988c	Rooney, 1988b	Rooney, 1989b
Formulation	A-20	B	MA-200	SP
Average Calf Weight	38.4	46.1	43	41.3
Dose, mg/kg/d	30.2	21.7	16.3	13.3
Withdrawal Day	Chlortetracycline, mg/kg of Liver Tissue			
0	16.7	1.82	6.5	13.7
15	NM	NM	0.073	NM
20	0.125	NM	0.075	NM
25	0.069	0.038	NM	ND-0.043
30	NM	ND-0.029	NM	NM
35	NM	ND-0.024	0.034	ND-0.036
40	NM	ND-0.029	NM	NM
45	ND-0.034	NM	NM	ND-0.025
55	NM	NM	NM	ND
60	NM	NM	ND-0.023	ND
65	ND-0.028	NM	ND	NM
75	ND	NM	NM	NM
	Chlortetracycline, mg/kg of Kidney Tissue			
0	25.3	2.18	9.7	19.2
15	NM	NM	1.09	NM
20	0.232	NM	0.092	NM
25	0.101	0.058	ND	0.059
30	NM	ND-0.039	NM	NM
35	NM	ND-0.030	0.069	0.058
40	NM	0.036	NM	NM
45	0.035	NM	NM	0.043
55	NM	NM	NM	0.031
60	NM	NM	ND-0.028	NM
65	0.033	NM	ND-0.028	ND-0.032
75	0.028	NM	NM	NM

Formulation: A-20 = AUROFAC 20 with neomycin and electrolytes in milk; B = CTC soluble boluses; MA-200 = AUROFAC 200-MA in milk replacer; SP = CTC soluble powder in milk replacer; NM = Not Measured; ND = Not Detected

Table 24. Depletion of Chlortetracycline (CTC) Residue From Liver and Kidney Tissues From Cattle Following Treatment with CTC in Feed

Reference	Gingher, 1981		Berger, 1965		Munger, 1978	
Average Weight, kg	208		458		318	
Days on CTC	14		61		28	
Dose, mg CTC/kg/d	22		11		4.4	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)					
	L	K	L	K	L	K
0	1.9	>5.0	0.99	2.53	0.52	1.37
3	NM	NM	0.18	0.56	NM	NM
5	0.05	0.17	NM	NM	NM	NM
10	ND	0.05	ND	0.05	ND	ND
14	0.05	0.05	NM	NM	ND	ND
21	ND	0.05	NM	NM	ND	ND

NM = Not Measured; ND = Not Detected (<0.025mg/kg)

Table 25. Depletion of Chlortetracycline (CTC) residues From Liver and Kidney Tissues from Cattle Following Treatment with AUREO S 700 in Feed

Reference	Berger, 1970	Colavita, 1967	Drain, 1966a	Drain, 1966b	Langner, 1976
Average Weight, kg	240	174	160	126	345
Days on CTC	94	29	33	30	28
mg CTC/kg/d	1.46	2.01	2.19	2.78	1.01
Withdrawal Day	Chlortetracycline, mg/kg of Liver				
0	ND-0.05	0.16	0.16	0.09	0.06
1	NM	NM	0.16	0.06	NM
2	NM	NM	0.05	NM	NM
4	NM	NM	ND	0.05	NM
7	ND	ND	ND	0.06	ND
10	ND	NM	NM	NM	ND
Withdrawal Day	Chlortetracycline, mg/kg of Kidney				
0	0.12	0.17	0.37	0.16	0.14
1	NM	NM	0.32	0.1	NM
2	NM	NM	0.1	NM	NM
4	NM	NM	0.04	0.08[1]	NM
7	ND	0.05	ND	0.10[1]	ND
10	ND	NM	NM	NM	ND

NM = Not Measured; ND = Not Detected (<0.025 mg/kg of tissue; [1]Contamination of withdrawal feed suspected, as control cattle tissues were initially negative, and then became positive at 4-7 day withdrawal points.

A series of experiments were conducted in which AUREO S 700[R] (a feed premix containing 77 g/kg of chlortetracycline and sulfamethazine) was fed to cattle for periods ranging from 28 to 94 days. Average daily dosages of chlortetracycline ranged from 1.01 to 2.78 mg/kg body weight in these studies (Table 25). Residue levels in liver and kidney were either less than the detection limit or were very low after seven days withdrawal of medicated feed (Berger, 1970; Colavita, 1967; Drain, 1966a,b; Langner, 1976).

Milk

Soluble bolus formulations of chlortetracycline are used for vaginal or intrauterine administration in cows for reproductive infections. A study was conducted in which four lactating Holstein cows received intrauterine administration of four chlortetracycline soluble boluses (2 grams chlortetracycline) as a single treatment 1 to 3 days postpartum. Average blood concentrations of chlortetracycline peaked at 0.149 mg/kg four hours after treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detected at 5 and 7 days post-treatment. Average levels of chlortetracycline in milk peaked at 0.146 mg/kg on day 1 post-treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detectable at 5, 6 and 7 days after treatment (Goodale, 1988a).

Residue data are available for two intramammary infusion products used for treatment of mastitis. The first study was conducted using an infusion product containing 426 mg of chlortetracycline per 6 mL syringe. One syringe was infused in each of the four quarters of the udder, and milk samples were assayed at 12 hour intervals until 120 hours post-medication. The 12-hour postmedication milk showed the highest activity, averaging 70 mg/kg chlortetracycline at that time. All milk samples were still positive at 96 hours post-treatment (average 0.07 mg/kg chlortetracycline). Four of the six cows still showed low activity (0.012 to 0.03 mg/kg) at the final sampling 120 hours post-treatment (Hewell, 1967). The second study was conducted with TARGOT[R] mastitis suspension containing 200 mg of chlortetracycline, 100 mg neomycin sulfate and 100 mg of dihydrostreptomycin sulfate (the latter two measured as base) per 6 mL syringe. One syringe was infused in each quarter of the udders of 10 clinically normal Dairy Friesians yielding approximately two gallons of milk daily. Individual cow milk samples were taken at 12-hour intervals for 144 hours after treatment. All milk samples contained less than 0.03 mg/L chlortetracycline at 120 hours after infusion and less than 0.125 mg/L dihydrostreptomycin sulfate-neomycin sulfate (combined assay) at 72 hours after treatment (Nelson, 1968).

Studies have shown that milk from cows receiving 0.22 mg chlortetracycline/kg bw daily by feed medication has no detectable chlortetracycline residues (Henderson, 1953; Sbor et al, 1959). When the feeding level of chlortetracycline was increased to 1.1 or 2.2 mg/kg bw daily, small amounts (up to 0.23 μg/mL) were found in the milk. After 48 hours withdrawal of medicated rations, all milk samples were again negative. The LOD of the assay was 0.01 μg/mL.

Ducks

One study was reported in which ducks were fed diets containing 400 mg/kg chlortetracycline for 3 weeks. At zero-day withdrawal of the medicated feed, average chlortetracycline residues of 0.39, 0.64, 1.95 and 0.04 mg/kg of muscle, liver, kidney and fat with skin, respectively, were found. After 5 days withdrawal, drug was not detected in fat with skin, but average chlortetracycline residues of 0.03, 0.06 and 0.24 mg/kg were still present in muscle, liver and kidney, respectively (Ferguson, 1972). Results are shown in Table 26.

Table 26. **Depletion of Chlortetracycline Residues in Ducks Receiving 400 ppm Chlortetracycline in Feed for 3 Weeks, (Ferguson, 1972)**

Withdrawal Day	Chlortetracycline, mg/kg of Tissue			
	Muscle	Liver	Kidney	Fat/Skin
0	0.39	0.64	1.95	0.04
1	0.1	0.18	0.6	ND
2	0.08	0.14	0.49	ND-0.011
3	0.04	0.1	0.35	ND-0.012
5	0.03	0.06	0.24	ND

ND = Not Detected

Sheep

Lambs were fed a fattening ration containing 50 mg/kg chlortetracycline (Kohler and Abbey, 1971) or 50 mg/kg chlortetracycline plus 50 mg/kg sulfamethazine (Wang, 1972a). A summary of the residue depletion data are shown in Table 27. Liver had no detectable residues of chlortetracycline after 4 days and kidney had no detectable residues of chlortetracycline after 8 days withdrawal from the medicated diets.

Table 27. **Depletion of Chlortetracycline (CTC) Residues in Liver and Kidney Tissues from Sheep After Having Received 50 ppm of CTC With and Without 50 ppm of Sulfamethazine (SMZ) in the Feed for 42 Days**

Reference	Kohler Abbey 1971				Wang 1971a			
CTC, ppm in Feed	50				50			
SMZ, ppm in Feed	0				50			
Withdrawal Day	CTC mg/kg							
	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat
0	0.027	0.11	0.33	ND	0.04	0.21	0.39	ND
2	ND	ND	ND-0.06	ND	NM	NM	NM	NM
4	ND	ND	ND	ND	ND	ND	0.04	ND
6	NM	NM	NM	NM	ND	ND	0.05	ND
8	NM	NM	NM	NM	ND	ND	ND	ND

NM = Not Measured; ND = Not Detected (<0.027, <0.03, <0.028 and <0.025 mg/kg in muscle, liver, kidney and fat, respectively)

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Because of the wide spread use of chlortetracycline and related tetracyclines there is a large body of literature detailing analytical procedures to quantitate residues. Most published methods in food matrices measure either the total antimicrobial activity or the concentration of free chlortetracycline. The predominant methodology routinely used to determine tetracycline antibiotics is high-performance liquid chromatography (HPLC). The majority of HPLC methods which have been validated for chlortetracycline determination are equally valid for both tetracycline and oxytetracycline.

A comprehensive review of analytical methods for tetracyclines has recently appeared (Oka and Paterson, 1995). A brief summary of more widely used procedures appears here.

Pharmaceutical preparation of tetracycline antibiotics frequently contain small amounts of impurities such as 4-epitetracyclines, anhydrotetracyclines and 4-epianhydrotetracyclines. Although these impurities are microbiologically inactive, 4-epianhydrotetracycline has been identified as the cause of Fanconi-syndrom (Flimter, 1973; Fluop and Drapkin, 1965) and it is therefore preferable to precisely quantitate tetracycline impurities during analysis.

The validated limit of chlortetracycline quantification (LOQ) in residue work reported above has been established at 0.020 mg/kg (20 ppb) in a variety of tissues. No interferences are experienced from typical veterinary products used in animal production.

High Performance Liquid Chromatograpby (HPLC)

Most of the successful chemical methods for reliable tetracycline analysis reported during the last 25 years are complex. Such complexity is imposed by the ease with which tetracyclines form chelate complexes with metal ions and the propensity to bind strongly with both proteins in the analytical matrix and silanol groups of the separation columns used during analytical determination. These problems were minimised by the use of either ethylenediaminetetracetic acid and oxalic acid at each analytical step (Oka et al, 1985 and references therein). The majority of liquid chromatographic methods presently used for determination of residues of tetracycline antibiotics espouse the principles of the Oka method and details of variations on the method have been exhaustively reviewed (Oka and Paterson, 1995). A collaborative study on the determination of chlortetracycline, oxytetracycline and tetracycline in edible animal tissues has recently been conducted (MacNeil et al, 1995).

A number of HPLC procedures for the analysis of tetracycline antibiotics take advantage of their metal-chelating capacity as a method to clean up tissue extracts prior to HPLC determination. Thus, much of the data for the residue studies reported above were gathered using an HPLC procedure which incorporated a copper-treated column during the isolation of analyte from the tissue matrix (Guzman, 1990, 1991, 1993, Farrington et al, 1991). However, the major principles of the Oka methodology were retained. In this procedure, chlortetracycline residues were extracted from the porcine muscle, liver, kidney, abdominal fat and skin with adhering fat tissues with an aqueous succinate buffer solution at pH 4. The filtered tissue extract was then applied to a copper (II)-loaded chelating sepharose column. Chlortetracycline was eluted from the copper (II)-loaded column with EDTA/succinate buffer solution at pH 4 onto an Amberlite XAD-2 column. Chlortetracycline was eluted from this column with methanol and quantitated by high performance liquid chromatography (HPLC). Chromatographic separations are carried out on a LC-8 reverse phase column with detection by UV at 350 nm with quantification against an external chlortetracycline standard.

A similar HPLC analytical procedure has been developed and validated for other animal tissues including milk and eggs by laboratories in Germany. This analytical methodology is described in Bestimmung von Rückständen, prepared by the Department of Animal Health of Bavaria, Germany (R127). Based on to the similarity of this method to the methods developed by Cyanamid (M 2023 and M 2328), the use of the HPLC procedures in all animal tissues is supported.

A different approach to tetracycline analysis has been reported by Agasoster and Rasmussen (1992) using a combination of on-line dialysis and solid phase extraction for clean-up, followed by HPLC with post-column photochemical derivatisation which results in the quantitative convertion of the tetracycline into a highly fluorescent product.

Liquid Chromatography - Mass Spectrometry (LC-MS)

In order to obtain definite confirmation of the identity in tetracycline residue analysis the use of a LC-MS confirmation is advisable. The use of a volatile LC-eluent is manditory in such separations. The use of either polystyrene-divinyl benzene co-polymer LC packing materials or well end-capped alkyl bonded chromatographic packing manufactured from ultra-pure silica gel has been recommended (Oka et al, 1993). The volatile eluent was comprised of methanol-acetonitrile-0.001M trifluoracetic acid mixtures. The use of different ionisation techniques in LC-MS of tetracyclines has been recently reviewed but no single method suitable for routine confirmatory work appears superior to all others at present. Electrospray ionisation would be ideally suitable for tetrayclines and may well be the future ionisation technique of choice for LC-MS measurement for this and a number of other antibiotic classes.

Microbiological Determination of Chlortetracycline

Microbiological procedures have been developed for the determination of chlortetracycline residue in tissues of swine, cattle, poultry, as well as for milk and eggs (Mariano, 1989, 1991).

In general, antibiotic residues are detected by modified US FDA cylinder plate agar diffusion microbioassays. Data summarized in Table 28 have been generated using tissue samples from treated animals to compare the results obtained using the microbiological assay and the HPLC assay techniques. These data clearly support the comparability of the two techniques.

Although microbiological and chemical assays have been shown to be comparable for a particular tetracycline antibiotic, it is not possible quantitatively to utilise microbiological assays for tetracyclines because there is a significant microbiological activity difference between different antibiotics of this class. Chemical methods are required for positive identification and quantification. However, microbiological assays remain valuable for screening purposes.

Table 28. **Comparison of Microbiological Assay and HPLC Analysis for Chlortetracycline (CTC) Residues in Kidneys from Pigs which Received 300 to 400 mg/kg in Feed for 7 Days (Gingher, 1990d)**

CTC, mg/kg in feed		300	300	400	400
Assay Method		MB	HPLC	MB	HPLC
Withdrawal Day		Chlortetracycline, mg/kg of Kidney			
0	Average	2.29	1.925	2.69	2.255
	Range	1.45-3.35	1.029-3.023	1.64-3.15	1.362-2.773
3	Average	0.121	0.101	0.148	0.124
	Range	0.108-0.129	0.095-0.114	0.074-0.245	0.062-0.221
5	Average	0.087	0.068	0.107	0.082
	Range	0.072-0.124	0.051-0.097	0.077-0.153	0.055-0.111
7	Average	0.08	0.054	0.069	0.049
	Range	0.067-0.100	0.042-0.074	0.058-.087	0.039-0.060
10	Average	0.06	0.04	0.047	0.029
	Range	0.050-0.070	0.034-0.044	0.039-0.053	0.023-0.034
12	Average	0.041	0.024	0.047	0.031
	Range	0.029-0.070	<0.02-0.033	0.030-0.062	0.020-0.043
15	Average	0.038	0.023	0.048	0.03
	Range	0.032-0.050	<0.02-0.03	0.037-0.060	0.022-0.039
20	Average	ND-0.035	<0.02	0.035	0.023
	Range	ND-0.046	<0.02-0.029	0.031-0.040	<0.02-0.034

MB = Microbiological assay; HPLC = High Performance Liquid Chromatography; ND = Not Detected

APPRAISAL

Chlortetracycline was last evaluated by JEFCA at the 12th Meeting in 1968, together with oxytetracycline and tetracycline. At that time, maximum residue levels were recommended of 0.05 mg/kg in meat and eggs and 0.02 mg/kg in milk.

The predominant use of chlortetracycline is as a prophylactic added to animal feed. It is rapidly but only moderately well absorbed from the GI tract and is eliminated in both urine and faeces either unchanged or in a microbiologically inactive form. There is no evidence that chlortetracycline is significantly metabolised *in vivo* although some *in vitro* chemiccal isomerisation of the drug can occur, either in the animal and/or during isolation. Microbiological assay is therefore a satisfactory method to acquire depletion of chlortetracycline from tissues. Tissue depletion measurements conducted both by microbiological and chemical assay give very similar results. Differencies in microbiological potency of individual tetracyclines, however, requires a preference for chemical determination of residues for regulatory purposes. Since chlortetracycline undergoes minimal metabolism, it is the appropriate marker compound for determination of residues in tissues.

Serum level and residue studies indicate that tetracycline is both rapidly absorbed and quickly cleared from edible tissues following oral administration. Kidney and liver tissues in all species show the largest concentration of chlortetracycline both at the withdrawal from the medication, and at any time point during the withdrawal period. These are also the last tissues to clear chlortetracycline residues. When liver and kidney tissues are in compliance with any designated MRL, residues in muscle will be less than 10% those found in kidney and fat should not cause any problem. Either liver or kidney (or both) could be considered as target tissue. It is recommended that kidney is the preferred target tissue.

In pigs receiving prophylactic chlortetracycline at the dose of 400 mg/kg in feed for 7 days mean residue levels were at 3 and 5 days 0.15 and 0.11 mg/kg in kidney and 0.11 and 0.08 mg/kg in liver after withdrawal of medication. Alternative administration of 198 mg/kg chlortetracycline in drinking water for 5 days gave residue levels of 0.31 and 0.05 mg/kg in kidney and liver, respectively, 2 days after withdrawal of drug.

Calves on feed containing 20 mg/kg for 7 days attain average chlortetracycline levels of 0.12 and 0.04 mg/kg in kidney and liver, respectively, 15 days after withdrawal of medication. Alternative dosing of calves with 22 mg/kg chlortetracycline, either as a soluble bolus or in drinking water, for 2-10 days gave residue levels of 0.45 and 0.27 mg/kg, respectively in kidney and liver, 7 days after drug withdrawal.

Cattle given 22 mg/kg of CTC in feed had kidney and liver residue levels of 0.20 and 0.10 mg/kg, respectively, 5 days after withdrawal of medication. Lactating cows given a single intrauterine dose of chlortetracycline gave residue levels in milk which were less than 0.05 mg/kg 3 days after dosing. Milk contained average residues of 0.07 mg/kg 4.5 days after cessation of treatment with mastitis formulations given at 426 mg/kg for 5 days. A dose of 3 g given to a lactating cow by intrauterine infusion leads to residues of less than 0.15 mg/kg 84 hours post dosing.

Broilers chickens receiving 528 mg/L of chlortetracycline in water together with feed containing 200 mg CTC/kg for 3 days had average residue levels in kidney and liver of 0.5 and 0.09 mg/kg, respectively, 2 days after withdrawal of drug. Residues levels averaging 0.3 and 0.05 mg/kg respectively were found in kidney and liver of chickens, given 200 ppm chlortetracycline continously in feed, 1 day after withdrawal from drug. Residues in eggs were below 0.05 mg/kg immediately after withdrawal of drug administered at the rate of 120 mg/L in drinking water for 7 days but doses of 600 mg/kg in feed over the same period gave residue levels of 0.19 mg/kg at day 1 of withdrawal. Turkeys given feed containing 600 mg/kg of chlortetracycline gave residue levels averaging 0.4 and 0.1 mg/kg in kidney and liver, respectively, 4 days after withdrawal. Four days after cessation of treatment, residue levels averaging 0.4 and 0.1 mg/kg, respectively, were found in kidney and liver of turkeys dosed with 528 mg/L chlortetracycline in drinking water for 3 days.

Two limited residue depletion studies were reported in lambs fed continuously with a fattening ration containing 50 mg/kg of chlortetracycline. At zero withdrawal time, the kidney, liver, muscle and fat contained 0.33, 0.11, 0.027 and <0.025 mg/kg of chlortetracycline residues, respectively. No residues were detected in these tissues at 4 days withdrawal.

Maximum Residue Limits

In reaching its decision on MRLs for chlortetracycline (and tetracycline) the Committee considered the following:

- MRLs were recommended for oxytetracycline at the 36th meeting of the Committee for all species of 600 μg/kg in kidney, 300 μg/kg in liver, 100 μg/kg in muscle, 100 μg/kg in milk, 200 μg/kg in eggs, and 10 μg/kg in fat. These levels were the lowest detectable by validated antimicrobial methods;

- chlortetracycline and tetracycline have been allocated a group ADI of 0-3 μg per kg of body weight with oxytetracycline;

- modern analytical techniques allow much more sensitive and specific assays than those provided by antimicrobial assays;

- the recommended target tissues for residue analysis in cattle, pigs and poultry are kidney and muscle. Based on limited data, the kidney is the recommended target tissue for sheep; and

- the marker residue for all three substances is parent drug.

The Committee recommended the following temporary MRLs for (both) chlortetracycline (and tetracycline) in cattle, pigs and poultry, expressed as parent drug:

Muscle - 100 μg/kg
Liver - 300 μg/kg
Kidney - 600 μg/kg
Eggs (poultry) - 200 μg/kg

The Committee also recommended temporary MRLs for sheep liver and kidney of 300 μg/kg and 600 μg/kg, respectively, expressed as parent drug.

The following information is required for evaluation in 1996:

- The results of residue depletion studies in cattle, sheep, pigs and poultry to determine the rate of depletion of residues in milk (cows), fat (all species) and in muscle, liver, kidney and fat (sheep), treated in accordance with approved uses of these substances; and

- new and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in tissues and milk.

ADI and MRLs allocated to chlortetracycline, and tetracycline are the same as those previously allocated to oxytetracycline at the 36th meeting for the given tissues and species. Although the Committee realized that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to tetracyclines were defined as applying both to residues of individual tetracyclines and to the sum of combined tetracycline residues, including chlortetracycline, oxytetracycline and tetracycline.

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DICLAZURIL

First draft prepared by
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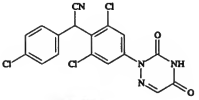
IDENTITY

Chemical name:	(±)-2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H-yl)benzeneacetonitrile
Synonyms:	CAS number 101831-37-2; Janssen Research Code R064433
Structural Formula:	



Diclazuril

Molecular Formula:	$C_{17}H_9N_4O_2Cl_3$
Molecular Weight:	407.6

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure Active Ingredient:	Diclazuril
Appearance:	Slightly yellow to beige colour
Melting Point:	292-297°C (dec)
Solubility:	Very low aqueous solubility (< 1 mg/l), 10 mg/l in 0.01N NaOH. Low solubility in most organic solvents (except dimethyl sulfoxide, N,N-dimethylformamide, and tetrahydrofuran). Octanol-water partition coefficient (log P) = 4.43 (pH = 3.0), 4.01 (pH = 4.98), 4.41 (pH = 7.03), and 4.48 (pH = 8.0). pKa = 5.92
UV_{max}:	275 nm

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RESIDUES IN FOOD AND THEIR EVALUATION

CONDITION OF USE

Diclazuril is a new anticoccidial drug intended for use in major poultry species including broiler chickens, replacement pullets and turkeys. It is also used in minor bird species, rabbits and lambs. For birds and rabbits, diclazuril is administered as a medicated feed premix at a recommended dose of 1 gram diclazuril per tonne (1 ppm). It is not intended for use in laying hens. It may be used in broiler chickens throughout the growing cycle but also may be used in a restricted manner designed to avoid the induction of resistance during the grow-out cycle as well. Diclazuril is used worldwide in poultry.

Lambs are treated with a 0.25% oral suspension at a 1 mg/kg bw dose when animals are 3-4 weeks old. Treatments may be repeated 2-3 weeks later. Registered use of diclazuril in lambs is currently limited but growing. Registered use in turkeys and rabbits is being developed.

PHARMACOKINETICS

Laboratory Species

A number of radiolabel drug studies were reported using ^{14}C-diclazuril in rats. In two early studies (5 animals each) with a 10 mg/kg bw oral dose in aqueous suspension, diclazuril residues were excreted rapidly (Meuldermans, W., et al., 1989a; Mannens, G., et al., 1992c). More than 90% of the radiolabel was excreted in faeces within 24 hours and approximately 97% in 96 hours. Most of the ^{14}C was identified as unchanged drug. Several minor metabolites were detected, however, the most prevalent metabolite accounted for less than 1% of the dose, indicating only minor metabolism in rats.

A ^{14}C-study on absorption and distribution was carried out in male rats using a 10 mg/kg bw aqueous oral suspension dose. Residue detection involved a gas chromatographic procedure with a limit of quantification of 0.05-0.2 mg/L in plasma (Van Beijsterveldt, L., et al., 1992d). Results indicated limited absorption as most of the radiolabel was eliminated by way of the gastrointestinal tract within 24 hours. Maximum plasma concentration of total residues and unchanged drug occurred at 8 h post dosing. The depletion was monophasic with a half-life of 53 h for total residues and 36 h for unchanged drug. On day 1, total residues in plasma were almost exclusively unchanged drug. Distribution to systemic tissues was rapid but limited. Maximum plasma concentrations of total residue and unchanged drug were approximately 1 mg/L at 8 hours post dosing. The $AUC_{0\text{-infinity}}$ (area under the curve) was 86.0 µg. h/L for total residues and 68.5 µg. h/L for unchanged parent drug. Results of the ^{14}C-radiolabel studies are summarized in Tables 1 and 2.

Table 1[1]. Total Residues (TR) and Unchanged Diclazuril (UD)(µg-eq/L) in Rat Plasma and Blood

Time (h)	Number of Animals	Blood	Plasma	
		(TR)	(TR)	(UD)
1	4	69 ± 7	104 ± 9	120 ± 13
2	4	200 ± 53	295 ± 68	323 ± 89
4	4	500 ± 87	710 ± 107	843 ± 113
8	4	723 ± 126	1035 ± 222	1213 ± 198
24	4	544 ± 59	822 ± 71	806 ± 75
48	4	450 ± 29	673 ± 61	578 ± 31
96	4	218 ± 67	332±110	215 ± 101

[1] = Data in all tables include mean value ± standard deviation unless noted otherwise.

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Table 2. Total Residues and Unchanged Diclazuril (mg-eq/kg) in Rat Tissues

Time (h)		1	2	4	8	24	48	96
Brain	TR	ND	ND	0.027	0.058	0.061	0.045	0.045
	UD	ND	0.017	0.037	0.052	0.043	0.029	ND
Heart	TR	ND	0.065	0.157	0.229	0.173	0.158	0.137
	UD	0.016	0.058	0.132	0.178	0.141	0.100	0.036
Lung	TR	0.024	0.066	0.191	0.252	0.263	0.183	0.170
	UD	0.038	0.083	0.178	0.229	0.227	0.125	0.056
Liver	TR	0.069	0.149	0.366	0.557	0.514	0.464	0.268
	UD	0.065	0.149	0.344	0.522	0.395	0.286	0.097
Kidney	TR	0.037	0.111	0.257	0.363	0.318	0.310	0.183
	UD	0.039	0.100	0.226	0.319	0.232	0.189	0.071
Muscle	TR	ND	ND	0.058	0.101	0.080	0.061	0.053
	UD	ND	0.018	0.053	0.073	0.057	0.042	0.013

ND = not detected.

A whole body autoradiography study in rats was reported using an oral dose of 10 mg/kg bw of ^{14}C-diclazuril. Results from this study were consistent with the data in Tables 1 and 2. Residues were highest in liver and lowest in muscle and fat.

Toxicokinetic studies in 160 Wistar rats (Monbaliu, J., et al., 1991b) and 160 Albino Swiss mice (Monbaliu, J., et al., 1991a) were carried out in support of 3-month subchronic oral toxicity studies using 1000, 2000 and 3000 ppm diclazuril in feed. Residues were measured in rat serum and mice plasma. Because of the growth of the rats and mice during the study, the estimated dose per kg b.w. decreased approximately 50% during the 3-month period. The average serum and plasma concentrations are summarized in Table 3.

Table 3. Average Serum Concentration of Diclazurilin Rats and Plasma in Mice (mg/L)

Dose (PPM)	Number of animals		Rats		Mice	
			male	female	male	female
1000	20M	20F	7.18	18.8	6.64	5.24
2000	20M	20F	13.1	30.1	8.85	8.92
3000	20M	20F	11.3	24.4	10.3	9.69

Although the plasma concentrations in male and female mice were similar, the serum concentrations were 2.2-2.6 times higher in female than in male rats. In both species, the systemic availability of diclazuril increased less than proportionally with the dose. The lack of linearity was attributed to absorption saturation at the high dose levels.

Rabbits

Two absorption, distribution, metabolism and excretion studies were conducted using 3 and 12 rabbits, respectively, (Meuldermans, W., et al., 1988c; Michiels, M., et al., 1988d) using a single oral dose in gelatin capsules with ^{14}C-diclazuril at 1 mg/kg bw. The excretion of radioactivity was fairly rapid. Within 48 h, 70% was recovered in the faeces and 3% in the urine. Within 10 days, more than 98% of the radioactivity was excreted (estimated at 91.3% in faeces and urine and 6.7% expired in air). Gall bladder contained only very low amounts of radioactivity, indicating that biliary excretion was a very minor pathway. Various metabolites were detected, although none accounted for more than 2% of the radioactivity. The two main metabolites were a glucuronide and a sulfate conjugate, based on comparison of radio-HPLC chromatograms from samples without enzymatic hydrolysis and with combination β-glucuronidase/arylsulfatase or arylsulfatase treatments. In the 12 animal study, radioactivity in plasma reached a plateau between 6 and 48 hours of 1.03-1.15 mg-eq/L and declined to 0.041 mg-eq/L after 240 h. The unchanged parent drug represented almost all the radioactivity up to 120 h after dosing. The elimination from plasma had an apparent half-life of 2-2.5 days. The distribution to tissues was limited with liver containing the highest concentrations. The half-life in liver was approximately 3 days. In muscle tissue, residues did not exceed 0.01 mg-eq/kg. Liver radioactivity was approximately 89-96% extractable. Tissue results from the 12 animal study using radio assay quantification procedures are summarized in Table 4.

Table 4. **Total Residues (mg-eq/kg) in Rabbits Dosed Orally With 1 mg/kg b.w. of Diclazuril**

Time (hours)	Liver	Kidney	Fat	Muscle
6	2.03 ± 0.23	0.88 ± 0.20	0.03 ± 0.01	0.01 ± 0.01
48	2.05 ± 0.77	1.12 ± 0.34	0.03 ± 0.03	0.05 ± 0.07
120	0.83 ± 0.24	0.29 ± 0.10	0.006 ± 0.005	0.005 ± 0.005
240	0.26 ± 0.07	0.05 ± 0.01	0.006 ± 0.005	ND

ND = not detected

Two tissue depletion studies using 16 rabbits (Michiels, M., et al., 1988a) and 48 rabbits (Van Beijsterveldt, L., et al., 1992f), respectively, also provide data on plasma kinetics. Each group of rabbits in these studies (equal numbers of male and females) received diclazuril medicated feed at a concentration of 1 mg/kg in feed for 14 days, using the commercial premix formulation. Taking account of the body weights and feed consumption of the rabbits, this gave an average intake of 0.067 mg/kg bw in the 16 rabbit study. Residues were determined using an UV-HPLC method. Steady state plasma concentrations (0.9-1.0 mg/L) were attained within 10 days of continuous dosing. The concentrations remained at this same level during the 24 h fast after removal of the medicated feed. Thereafter, diclazuril residues depleted with a half-life of about 2 days. Similar results were obtained in the 48 rabbit study. Plasma concentration 24 h post dosing was 0.67 ± 0.30 mg/L with a depletion half-life of 2.5 days.

Chickens

Three ^{14}C-diclazuril studies were reported in chickens. In the single dose study using a 1 mg/kg bw oral dose, maximum diclazuril concentrations of 1.5-2.0 mg-eq/L were observed in plasma at 6 h after administration, with an elimination half-life of about 50 h (Meuldermans, W., et al., 1988e). Plasma radioactivity was completely recovered in the supernatants after removal of proteins with acetonitrile. Radioactivity originated almost exclusively in the unchanged drug for up to 72 h. Equilibrium between plasma and tissues was rapid, with tissue concentrations 2-10 times lower than the plasma concentration. The half-life in tissues was about 50 h. Extraction of liver radioactivity in methanol/formic acid (100/0.5; v/v) was virtually quantitative. Diclazuril accounted for more than 90% of the residues in the 24 h liver samples with metabolites being less than 4% of the residues. After 10 days the cumulative excretion was greater than 95%. An excreta degradation product accounting for 5.3% of the residues was attributed to a derivative of 4-amino-2,6-a-(4-chlorophenyl)-benzeneacetonitrile formed by cleavage and further degradation of the 1,2,4-triazine-dione ring. Other metabolites accounted for less than 2% each of residues and were not identified. Results are summarized in Table 5.

Table 5. **Total ^{14}C Diclazuril Residues (mg-eq/L or mg-eq/kg) in Chickens Receiving a Single Oral 1 mg/kg BW Dose**

Time (hours)	Plasma	Liver	Kidney	Muscle (PM)	Muscle (FM)	Skin/Fat
6	1.7±0.24	1.26±0.18	1.07±0.17	0.15±0.03	0.17±0.04	0.14±0.02
24	1.30±0.23	0.92±0.12	0.73±0.12	0.12±0.02	0.11±0.04	0.11±0.03
48	1.10±0.10	0.79±0.05	0.63±0.05	0.10±0.01	0.06±0.04	0.09±0.02
72	0.74±0.16	0.42±0.06	0.36±0.07	0.05±0.03	0.06±0.02	0.06±0.01
120	0.45±0.12	0.28±0.10	0.23±0.05	0.05±0.02	0.05±0.02	0.05±0.02
168	0.21±0.12	0.09±0.05	0.12±0.06	0.02±0.01	0.02±0.00	0.03±0.03
240	0.08±0.04	0.03±0.00	0.05±0.02	0.01±0.00	0.01±0.01	0.01±0.00

PM = pectoral muscle; FM = femoral muscle.

One multiple dosing study was conducted in chickens and described in two separate reports (Michiels, M., et al., 1987; Meuldermans, W., et al., 1989b). In addition to drug absorption, excretion and residues in tissues following multiple dosing to chickens, the study reported on the mass balance of unchanged drug and metabolites in plasma, tissues and excreta. ^{14}C-diclazuril was given to broiler chickens for 14 days (from day 28 to 41 days of age) with an average daily dose of 0.090 mg/kg bw, divided into 2 treatments per day orally in a gelatin capsule. Total radioactivity depleted at similar rates from plasma and tissues with a half-life of about 2.5 days. The highest steady state concentration in plasma was 589 ± 49 µg-eq/L. The residues were determined using the gas chromatography-electron capture detector (GC-ECD) method. The extraction procedure recovered 88% of residues from plasma and more than 80% from tissue. Metabolic profiling studies using radio-HPLC confirmed that all plasma radioactivity was from parent drug. In plasma, muscle and skin/fat the total residue (TR) was unchanged drug (UD); in liver UD was also the main radiolabel compound. Mass balance degradation product studies after single and multiple dosing indicate the main metabolite in the excreta accounted for 5.6-8.3% of the dose. Results are summarized in Tables 6 and 7.

Table 6. **Plasma Residues in Chickens (µg-eq/L) from 0.045 mg/kg bw b.i.d. 14 Day Treatment with Diclazuril in a Capsule Formulation eq. to 1 ppm in Feed**

Time (hours)	6	24	72	120	168	240
TR	589±49	316±54	226±31	138±53	64±31	34±16
UD	608±51	321±57	224±23	53±23	65±31	34±14

A whole-body autoradiography study with five birds dosed at 0.5 mg/kg bw by gavage into the crop gave similar results to those listed in Table 7. Birds were sacrificed at 6, 24, 48, 72 and 120 h post dosing. In tissues, the highest radioactivity was observed in the liver, kidney, lung, connective tissue and skin; the lowest concentrations were in muscle, brain and fat. There was a similar distribution and concentration decrease as a function of time for blood and tissues (Michiels, M., et al., 1987b).

Table 7. Tissue Residues in Chickens (μg-eq/kg) from 0.045 mg/kg bw b.i.d. 14 Day Treatment with Diclazuril in a Capsule Furmulation eq. to 1 ppm in Feed

Time (h)/ Tissue		6	24	72	120	168	240
Liver	TR	386±69	240±57	187±31	107±17	63±12	36±5
	UD	370±52	202±53	138±21	85±29	42±16	20±7
Muscle (PM)	TR	58±5	31±6	23±2	13±4	7±3	< 5
	UD	52±5	27±5	19±2	13^1	< 10	< 10
Muscle (FM)	TR	87±7	44±5	32±5	19±6	10^2±5	6±2
	UD	72±8	37±5	25±3	16^1	< 10	< 10
Skin/Fat	TR	193±17	110±16	83±11	49±10	29^1±12	17±8
	UD	158±22	85±13	59±5	41±13	22^1±8	< 10^2
Kidney	TR	324±38	199±38	133±20	79±21	41±14	23±8
	UD	NR	NR	NR	NR	NR	NR

PM = pectoral muscle; FM = femoral muscle; ^{1}n = 7 birds (all other groups 8 birds); ^{2}= median value; NR = not reported

Turkey

Two radiolabel studies were reported in turkeys using ^{14}C-diclazuril. In the single oral dose study (1 mg/kg bw) using 28 birds (Meuldermans, W., et al., 1990a), the highest residues were found in plasma 6 h post dosing with subsequent elimination proceeding with a half-life of approximately 38 h. Results were similar to those in chickens. The plasma radioactivity could be fully recovered in the deproteinized supernatants and was almost exclusively due to unchanged drug for up to 168 h. There was a rapid but limited distribution between plasma and tissues. Depletion half-lives in tissues ranged from 34-46 h. In liver, extraction of radioactivity in methanol/formic acid (100/0.5; v/v) was nearly quantitative. Radiochromatograms of liver tissue indicated that unchanged drug accounted for 98% of sample radioactivity at 6 h and 85% after 48 and 72 h. No metabolites accounted for more than 10% of the radioactivity in liver. At least eight metabolites were detected in the excreta extracts. The triazine-dione ring cleavage product seen in broilers accounted for 6.3% of the dose in the 0-96 h excreta. A second unidentified metabolite accounted for 2.4% and all others were less than 2% each. The radio-assay method in this study had a quantification limit of 0.01 mg/kg or mg/L. Residue data are summarized in Table 8. Standard deviations are available but not recorded here.

In the 12 turkey multiple dosing study, ^{14}C-diclazuril was given orally to male and female turkey poults approximately 11 weeks old for 14 days (Byrd, J. and Lucht, K., 1992e). The diclazuril dose was approximately 0.05 mg/kg bw divided over two treatments per day in a gelatine capsule. All birds were sacrificed 6 h after the last dose. Extraction using ethyl acetate recovered 89.1% of the radioactivity in livers of male turkeys and 95.7% in female turkeys. Male and female turkey livers contained minor additional peaks, but none representing more than 4% of the total residues or concentrations exceeding 0.015 mg-eq/kg. In the male liver tissue, total residues were 0.36 mg-eq/kg with 0.26 mg-eq/kg being unchanged drug. Two metabolites accounted for 0.014 and 0.013 mg-eq/kg, respectively. Nonspecified extracted total residues (peaks in the radiochromatograms which were not seen consistently between replicates) accounted for 0.04 mg-eq/kg and 0.04 mg-eq/kg unextracted total residues. In females, the respective values are 0.58 mg-eq/kg total residues, with 0.48 mg-eq/kg being unchanged drug, one metabolite at 0.016 mg-eq/kg, nonspecified extracted total residues at 0.06 mg-eq/kg and unextracted total residues of 0.03 mg-eq/kg. Characterization of liver residues noted above employed a radio-HPLC method. Average tissue concentrations summarized in Table 9 were determined by a combustion-scintillation radio-assay.

Table 8. **Residues of Diclazuril in Turkeys (mg-eq/kg or mg-eq/L) Receiving a Single 1 mg/kg bw Oral Dose**

Time (h) Tissue		6	48	72	120	168	240
Plasma	TR	1.78	0.85	0.46	0.18	0.10	0.03
	UD	1.36	0.77	0.39	0.13	0.08	0.02
Liver	TR	1.40	0.71	0.36	0.16	0.12	0.04
	UD	1.35	0.55	0.25	0.09	0.05	0.01
Kidney	TR	1.09	0.45	0.24	0.09	0.05	0.01
	UD	0.88	0.44	0.21	0.07	0.05	ND
Muscle (PM)	TR	0.21	0.08	0.04	0.02	0.01	ND
	UD	0.16	0.07	0.04	0.02[1]	ND	ND
Skin/Fat	TR	0.57	0.21	0.15	0.04	0.02	0.01
	UD	0.21	0.11	0.07	0.04	0.02	0.01[1]

PM = pectoral muscle; ND = not detected; [1] = Median value reported.

Table 9. **Average Tissue Concentrations (mg-eq/kg) in Turkeys Six Hours Following Last Dose in a 14 Day Oral Dosing Study (Byrd, J. and Lucht, K., 1992e)**

Tissue	TR in mg-eq/kg		Average % of dose	
	Males	Females	Males	Females
Breast muscle	0.049	0.062	1.44	1.44
Thigh muscle	0.070	0.088	1.22	1.40
Abdominal fat	0.186	0.307	0.31	0.62
Kidney	0.304	0.439	0.22	0.22
Liver	0.407	0.610	1.29	0.21

Sheep

There are no *in vivo* radiolabeled studies in sheep, however, there was a report concerning the comparative *in vitro* metabolism. Two non-radiolabeled studies were reported. In the first, 3 sheep were treated with a 1 mg/kg bw oral drench as a 0.25% suspension as intended for registered use. Poor systemic absorption of diclazuril was noted (Monbaliu, J., et al., 1993). Maximum concentrations in plasma were 0.012-0.016 mg/L at 24 to 48 h after dosing, and were below the limit of quantification (0.01 ug/ml) at all other sampling times. Plasma kinetics were evaluated as part of a residue depletion study where six lambs per group were dosed orally at 1 mg/kg bw with a 0.25% oral suspension. One group was dosed at 4 weeks of age and a second group twice, once at 4 weeks and once at 7 weeks of age (Van Beijsterveldt, L., et al., 1994b). Bioavailability was higher in the younger animals used in the second study. In the second study, the maximum plasma concentration was observed at 24 h post treatment for the first dose (0.15 ± 0.10 mg/L) and 0.08 ± 0.02 mg/L following the second dose. The area under the curve (AUC) calculations were 10.5 ± 7.5 mg.h/L and 4.89 ± 1.51 mg.h/L for the single and double treatment groups of sheep, respectively. Half-lives were estimated at 30.6 ± 5.9 h for the first study group and 28.0 ± 8.6 h for the second group of sheep. Residue concentrations are summarized in Tables 10 and 11.

Table 10. Residue Concentrations in Four Week Old Sheep (mg-eq/kg) after a Single Oral 1 mg/kg bw Treatment

Days Post Treatment	Plasma	Liver	Kidney	Muscle	Fat
1	0.14±0.05	0.30±0.10	0.09±0.03	0.03±0.01	0.08±0.03
3	0.04±0.03	0.11±0.07	0.03±0.02	≤ 0.01	0.03±0.01
5	0.01±0.01	0.03±0.03	≤ 0.01	≤ 0.01	0.04±0.05
7	≤ 0.005	0.02±0.01	≤ 0.01	≤ 0.01	≤ 0.01

Table 11. Residue Concentrations in Seven Week Old Sheep (mg-eq/kg) after Two Oral 1 mg/kg bw Treatments

Days Post Treatment	Plasma	Liver	Kidney	Muscle	Fat
1	0.07±0.04	0.28±0.19	0.04±0.02	0.01±0.01	0.04±0.02
3	0.02±0.03	0.06±0.02	0.01±0.01	≤ 0.01	0.01±0.00
5	≤ 0.005	0.02±0.03	≤ 0.01	≤ 0.01	≤ 0.01
7	≤ 0.005	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01

Depletion half-lives in tissue and plasma were similar.

TISSUE RESIDUE DEPLETION STUDIES

Residue depletion studies of diclazuril from the edible tissues of rabbits, broiler chickens, turkeys and lambs were conducted using conditions simulating normal treatment and husbandry practices.

Rabbits

Two studies were reported in rabbits using diclazuril at 1 mg/kg in their feed for 14 days (Michiels, M., et al., 1988a; Van Beijsterveldt, L., et al., 1992f). Concentrations of diclazuril were determined using an HPLC-UV method with a limit of quantification (LOQ) of 0.05 mg/L in plasma and 0.1 mg/kg in tissue. Steady state concentrations were reached within 10 days of continuous feeding. The plasma concentration at steady state conditions was 0.89±0.17 mg/L. In both studies the depletion half-life was 2-2.5 days for plasma and kidney. For liver the half-life was 3.9 days. Results are summarized from both studies in Table 12.

Table 12. Tissue Residues in Rabbits (mg-eq/kg) Following Fourteen Days Multiple Dosing

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1[1]	1.59±0.26	0.64±0.26	< 0.10	<0.21±0.18	1.02
7[1]	0.71±0.26	<0.16±0.07	< 0.10	< 0.10	0.23±0.15
1	1.45±0.30	0.44±0.10	0.05±0.03	ND	0.67±0.30
3	0.84±0.34	0.22±0.15	ND	ND	0.30±0.22
5	0.70±0.28	0.13±0.08	ND	ND	0.17±0.11
7	0.51±0.26	0.08±0.08	ND	ND	0.10±0.09
10	0.27±0.15	ND	ND	ND	0.05±0.07

[1] = Non-GLP study; ND = not detected.

Chickens

Two medicated feeding studies were reported with birds receiving diclazuril at 1 mg/kg medicated feed for a complete grow out (46 days). In the first study (Van Leemput, L., et al., 1989c) diclazuril was administered using the European type premix containing 0.5% of active ingredient. Residues in this study were determined using an HPLC-UV method with a LOQ of 50 µg/kg for tissues and 50 ng/ml for plasma, except skin/fat which had a LOQ of 100 µg/kg. In the second study (Van Leemput, L., et al., 1990b), the U.S. premix containing 0.2% of active ingredient was used with residues determined using a GC-ECD method with a LOQ of 10 µg/kg and 10 ng/ml for tissues and plasma, respectively. Sampling for residue analysis was based on selection of 10 birds from each treatment group (of 50 birds each). The two lightest and heaviest birds were eliminated from each group of 10, and the remaining six birds analyzed individually. Half-lives in tissues using the European premix were 34 h in kidney, 44 h in liver, 50 h in muscle and 52 h in plasma. Using the U.S. premix, half-lives were 61 h in kidney, 58 h in liver, 59 h in muscle, 65 h in plasma and 65 h in skin/fat. Results are summarized in Tables 13 and 14.

Table 13. Residues in Broiler Chickens (µg-eq/kg) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the European Premix Containing 0.5 Percent Diclazuril (46 Day Treatment Period)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	419±26	517±40	91±27	ND	951±123
72	154±19	186±94	ND	ND	430±100
120	92±17	64±52	ND	ND	259±79
168	ND	ND	ND	ND	129±40
216	ND	ND	ND	Nd	74±58

ND = not detected.

Table 14. Residues in Broiler Chickens (μg-eq/kg) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the U.S. Premix Containing 0.2 Percent Diclazuril (46 Day Treatment Period)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	371±90	308±65	45±9	144±35	565±168
24	340±97	268±92	39±11	138±39	476±157
48	180±80	146±76	23±10	79±35	267±178
72	184±90	184±131	26±15	91±49	347±266
96	93±19	73±12	12±3	42±6	152±34
120	100±36	85±28	13±5	48±12	158±57
168	51±23	44±24	ND	23±11	95±57
216	33±9	30±8	ND	18±5	69±19

ND = not detected.

Turkey

For turkeys, two 16 week feeding studies were reported (320 birds each) using 1) the European premix (0.5%) at a 1 mg/kg final concentration in medicated feed, and 2) the U.S. premix (0.2%) at a 1 mg/kg final concentration in medicated feed (Van Beijsterveldt, L., et al., 1992a; Van Beijsterveldt, L., et al., 1992b). For residue analysis, turkeys were sampled as noted above for broiler chickens, using 160 turkeys as controls in both studies. Samples were frozen quickly after collection and analyzed within 2-3 months using a reverse-phase HPLC-UV procedure with a LOQ of 50 ng/ml for plasma, 50 μg/kg for liver, muscle and kidney, and 100 ug/kg for skin/fat for the 1990 study with the European premix and a GC-ECD procedure with a LOQ of 10 μg/L in plasma and 10 μg/kg in tissue in the 1992 study with the U.S. premix. Depletion half-lives were 3 days in plasma, liver, muscle and kidney. Results are summarized in Tables 15 and 16.

Table 15. Residues in Turkeys (mg-eq/kg or mg-eq/L) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the European Premix Containing 0.5 Percent Diclazuril (16 Week Treatment)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	0.57±0.05	0.30±0.02	ND	0.16±0.05	0.80±0.12
24	0.37±0.025	0.18±0.02	ND	0.18±0.05	0.65±0.06
72	0.25±0.02	0.07±0.03	ND	0.11±0.06	0.41±0.05
120	0.17±0.02	ND	ND	ND	0.26±0.03
168	0.15±0.05	ND	ND	ND	0.16±0.03
216	0.09±0.03	ND	ND	ND	0.11±0.02

ND = not detected.

Table 16. Residues in Turkeys (mg-eq/kg or mg-eq/L) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the U.S. Premix Containing 0.2 Percent Diclazuril (16 Week Treatment)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	0.40±0.04	0.29±0.01	0.05±0.01	0.15±0.02	0.57±0.02
24	0.30±0.03	0.27±0.02	0.04±0.05	0.13±0.02	0.52±0.03
48	0.26±0.02	0.22±0.02	0.03±0.00	0.12±0.01	0.44±0.05
72	0.19±0.01	0.16±0.01	0.02±0.00	0.10±0.01	0.33±0.05
120	0.12±0.01	0.10±0.01	0.02±0.00	0.08±0.02	0.18±0.02
168	0.08±0.02	0.06±0.01	ND	0.07±0.02	0.11±0.02
216	0.05±0.01	0.04±0.00	ND	0.05±0.01	0.08±0.00

ND = not detected

Sheep and Lamb

Two residue studies were reported in lambs using the recommended 0.25% suspension to provide a dose of 1 mg/kg bw. The first study was not done under GLP conditions. This study did not describe the HPLC method used for residue analysis except noting the LOQ in tissue (0.05 mg/kg for liver, kidney and muscle, and 0.10 mg/kg for fat). In all tissues at all post treatment times (24 h, 3 and 7 days) no residues were detected. In the second study (Van Beijsterveldt, L., 1994b), lambs were treated with a 0.25% suspension at 0.4 ml/kg (1 mg/kg bw) by gavage. Dosing was done once, at 4 weeks of age, or twice, at 4 and 7 weeks of age. Edible tissues were collected from 6 animals at each post treatment time. Residues were determined using a validated GC method with a LOQ of 5 μg/L for plasma and 0.01 mg/kg in tissue. Blood samples were collected also and the plasma analyzed for residues of diclazuril. Edible tissues were collected from 6 animals each at 1, 3, 5, and 7 days after treatment (for the single and repeat dosing groups). Maximum concentrations in plasma following a single dose was 0.15 ± 0.10 mg/L, the maximum concentration occurred at 21 ± 7 h, and the half-life was 30.6 ± 5.9 h. Results following the second dose treatment was 0.08 ± 0.02 mg/L, the maximum drug concentration occurred at 24 ± 0 h, and the half-life was 28.0 ± 8.6 h. Depletion half-lives in tissue and plasma were similar. Results are summarized in Tables 17 and 18.

Table 17. Residues in Lambs (mg/L or mg/kg) after a Single 1 mg/kg bw Treatment with a Diclazuril 0.25% Drench at the Age of 4 Weeks

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1	0.30±0.10	0.09±0.03	0.03±0.01	0.08±0.03	0.14±0.05
3	0.11±0.07	0.03±0.02	≤ 0.01	0.03±0.01	0.04±0.03
5	0.03±0.03	≤ 0.01	≤ 0.01	0.04±0.05	0.01±0.01
7	0.02±0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005

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Table 18. Residues in Lambs (mg/L or mg/kg) after a Single 1 mg/kg bw Treatment with a Diclazuril 0.25% Drench at the Age of 4 and 7 Weeks

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1	0.28±0.19	0.04±0.02	0.01±0.01	0.04±0.02	0.07±0.04
3	0.06±0.02	0.01±0.01	≤ 0.01	0.01±0.00	0.02±0.01
5	0.02±0.03	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005
7	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005

Eggs

Two studies evaluated possible diclazuril residues in eggs. In the first study, 180 laying hens were given medicated feed at 1 or 5 mg/kg for 32 days to simulate a misfeeding or an overdosing in a non-target species. Eggs were collected during the dosing period and for 20 days following withdrawal of medicated feed. Residues were 3.7-3.9 times higher in egg yolk than in egg whites and 4.1-4.4 times higher after the 5 mg/kg than the 1 mg/kg dose (Van Leemput, L., et al., 1990e). Residues were determined using a GC method with a LOQ of 0.05 mg/kg. Steady state concentrations were noted in egg white after 11 treatment days and 14 days for egg yolk. The residue depletion half-life was 4-6 days. Average steady-state concentrations were 0.065 mg/kg in egg white and 0.24 mg/kg in egg yolk, equivalent to about 7.1 μg in a whole egg.

In the second study, 20 replacement pullets were maintained on 1 mg/kg diclazuril medicated feed until 16 weeks of age (Van Leemput, L., 1994a). The young hens were kept subsequently on a non-medicated feed and, on the onset of laying, all eggs from the first collection were sampled. Residue analysis using a GC method with a LOQ of 0.05 mg/kg indicated that none of the egg samples contained diclazuril at concentrations above the LOQ.

MARKER RESIDUE AND TARGET TISSUE

The radiolabel studies in laboratory and food producing animals measured total diclazuril related residues in edible tissues and characterized total residue depletion up to 240 hours post administration (depending on species). Diclazuril is metabolized to a very limited extent in all species tested. No study indicated more than 10% of total residues being diclazuril metabolic products. The studies also verified nearly quantitative extraction of diclazuril related residues, indicating minimal bound residues in edible tissue over all time periods studied. This is particularly evident in the 6-24 h residue data. These data support parent drug, diclazuril, as the appropriate marker residue.

The highest residue concentrations are consistently found in liver and kidney, with significantly lower residues in fat for red meat animals and skin/fat for poultry. Residues are lowest in muscle tissue for all species. For practical reasons, liver is the first tissue of choice for residue analysis. Concentrations of residues are higher in skin/fat or fat than in muscle tissue. Muscle tissue is, however, more convenient and more appropriate for residue analysis than skin/fat or fat tissue in international trade. A summary of residue data from studies using the recommended treatments is presented in Table 19.

Table 19. **Mean and Maximum Observed Diclazuril Residues (mg/kg) in Food Animals Using Recommended Treatments[1]**

Species		Liver	Kidney	Muscle	Skin/Fat	Fat
Rabbit	mean max.	1.59 1.93	0.64 0.90	< 0.10 < 0.10		< 0.21 0.38
Rabbit	mean max.	1.45 1.87	0.44 0.55	0.05 0.09		0.05 0.23
Chicken	mean max.	0.42 0.45	0.52 0.59	0.09 0.13	< 0.10 0.14	
Chicken	mean max.	0.37 0.50	0.31 0.39	0.05 0.06	0.14 0.19	
Turkey	mean max.	0.57 0.64	0.30 0.34	0.05 0.08	0.16 0.26	
Turkey	mean max.	0.40 0.44	0.29 0.29	0.05 0.05	0.15 0.17	
Lamb	mean max.	0.30 0.61	0.09 0.15	0.03 0.05		0.09 0.13

[1] Maximum residue concentration of diclazuril is at 6 h post treatment for chickens and turkeys, 24 h for rabbits and lambs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Three methods were reported for analysis of diclazuril residues - gas chromatography using a packed column with an electron capture detector (Meuldermans, W., et al., 1989b), gas chromatography using a capillary column with a thermionic specific detector (Corbin, T., 1990c; Corbin, T., 1990d), and a reverse-phase high performance liquid chromatography with UV detection (Woestenborghs, R., 1988h).

The capillary GC method was designed for use as a regulatory method for residues in chicken liver. The method involves extraction of 10 g of ground liver with ethyl acetate and clean-up with solid phase extraction over C-18 reversed-phase cartridges. Purified extracts are derivatized with pentafluorobenzylbromide. A specific thermionic detector is coupled with the chromatograph, using a 15 m x 0.25mm i.d. column coated with DB-1301. An internal standard (R062646) is used as an aid for quantification. Average recoveries were about 78% with analyte linearity from 25-250 μg/kg. The method has good specificity and a limit of detection of 20 μg/kg.

The GC-electron capture detection method is suitable for residues in plasma and edible tissue of broilers, turkeys and lambs as well as egg yolks and egg whites. The procedure requires homogenization of samples of muscle, liver or kidney. Aliquots of the homogenates are fortified with the internal standard noted above and residues are extracted with ethyl acetate. The extract is evaporated to dryness and redissolved in acetonitrile:water (1:1, v/v), washed with hexane and extracted again with ethyl acetate. The organic layers are evaporated to dryness. Samples of skin/fat or fat were ground and homogenized (1:9, v/v) in 0.1M citric acid. The homogenate is extracted with ethyl acetate, evaporated to dryness and redissolved in 80:20 (v/v) acetonitrile-water, washed three times with hexane and extracted with ethyl acetate and evaporated to dryness. The dry residues are dissolved in diazomethane solution, allowed to react for 5 minutes at room temperature, evaporated to dryness and redissolved into toluene for GC analysis. Chromatography uses a glass column (1 m x 4 mm i.d.) packed with 3% OV-17 on 80-100 mesh Supelcoport. Detection used a ^{63}Ni electron capture detector.

Performance characteristics for the GC-ECD method were reported. The limit of quantitation in animal tissues is 0.01 mg/kg and 0.025 mg/kg for egg whites and yolks. The linear range is 0.01-2.0 mg/kg with an accuracy of 92-109% and precision of 0-7.8%. For eggs, linearity was demonstrated at 0.05-0.5 mg/kg. For egg yolk at 0.25 mg/kg, the accuracy was 78% with precision of 10.8%; for egg white at 0.1 mg/kg, the accuracy was 84% and precision was 14.1%. The method meets attributes suitable for regulatory purposes.

The HPLC method is applicable for plasma and edible tissues of broilers, turkeys and rabbits (Woestenborghs, R., 1988b). Tissues are homogenized in distilled water (1:4, v/v), and aliquots fortified with an internal standard (R062370). Residues are extracted with ethyl acetate, evaporated to dryness, redissolved in 50:50 (v/v) acetonitrile-water, washed with hexane and reextracted with ethyl acetate. The evaporated extract is reconstituted in HPLC elution solution and chromatographed on a reverse-phase 10 cm x 4.6 mm i.d. column packed with 5 μm particle size ODS-Hypersil using 0.1M ammonium acetate-tetrahydrofuran (69:31, v/v) and UV detection at 278 nm. The LOQ is 0.05 mg/kg in tissues. Some interferences can occur in fat and skin/fat samples and the method is not recommended for fatty samples with residues below 0.5 mg/kg. The linear range is 0.04-10 mg/kg in tissues and 0.08-20 mg/kg in fat. In these conditions, the accuracy is 78-117% with a precision of 1.4-16.3%. Overall, it is less suitable than the GC-ECD method because of inferior LOQ's and possible interferences in fatty samples.

APPRAISAL

Diclazuril is a relatively new anticoccidiostat drug. As such, almost all relevant studies have been performed under good laboratory practices. Published literature dates from 1985. Diclazuril pharmacokinetics have been studied in laboratory and food animal species. Residue and metabolism studies in edible tissues of broilers, turkeys, sheep and rabbits were conducted as recommended for the practical use of diclazuril.

Diclazuril is generally well absorbed in all species investigated, however, it may be somewhat less well absorbed in sheep than in lambs. There were no indications of significant metabolism of diclazuril. Residue based substances were eliminated mainly as unchanged drug in the excreta of bird species and the faeces of rats and rabbits. Radiolabel studies yield almost quantitative extraction of residues indicating no significant bound residues in any species. In edible tissues of food animals, the residues consisted almost exclusively of parent drug, particularly at short withdrawal times (6 h for poultry, 24 h for sheep and rabbits). Metabolites at these withdrawal times were less than 10% of total residues in all tissues. The lack of metabolism is a dominant feature in all species, particularly for short withdrawal times. The common identifiable metabolite among species indicates that there are no significant species differences in the metabolism of diclazuril. These data also support the selection of diclazuril as the marker residue compound for compliance purposes. Liver and muscle are the tissues of choice for residue analysis.

Diclazuril residues are dependent on the mode of administration. Poultry fed at 1 mg/kg bw in feed for prolonged periods (e.g., complete grow out) results in residues of approximately 0.6 mg/kg or less in liver and kidney tissues at 6 h withdrawal. The residues are lower in skin/fat and lowest in muscle tissue. In rabbits, residues are higher in liver and kidney than in poultry but exhibit the same general pattern, with residues lowest in muscle tissue. Treatment of lambs at 1 mg/kg bw oral dosing results in high liver, but relatively low residues in kidney, fat and muscle tissue. For broilers, turkeys and rabbits, the relative distribution of residues in edible tissues did not change with the time after last treatment. Depletion from all tissues proceeded with the same approximate half-life.

Analytical methods are capable of quantitating residues in all species and tissues at concentrations of interest. The GC-ECD procedure has a limit of quantitation of 0.010 mg/kg in tissues of broilers, turkeys and lambs (as well as egg white and yolk), whereas the HPLC method has a limit of quantitation of 0.04-0.05 mg/kg in edible tissues of broilers, turkeys and rabbits.

Maximum Residue Limits

Based on the temporary ADI of 0-20 μg per kg body weight established by the Committee using toxicological data, the permitted daily intake of diclazuril residues would be 1200 μg for a 60 kg person.

In recommending the temporary MRLs, the Committee took into consideration that two regulatory methods are available for residue analysis. The gas chromatography-electron capture detection method which has a limit of quantification of 0.01 mg/kg and a high performance liquid chromatography method using ultraviolet detection which has a limit of quantification of 0.05 mg/kg. The Committee also took into consideration the maximum residues observed in poultry, sheep and rabbits when diclazuril is used according to good veterinary practices.

The recommended temporary MRLs, expressed as parent drug, in poultry, sheep and rabbits, are shown in Table 20.

Table 20. Temporary MRLs (μg/kg) For Poultry, Sheep and Rabbit

Species	Tissue			
	Muscle	Liver	Kidney	Fat
Poultry	500	3000	2000	1000
Sheep	500	3000	2000	1000
Rabbit	500	3000	2000	1000

If these values are used for temporary MRLs, the theoretical intake of residues of diclazuril in all species listed is 600 μg, using a daily food intake of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat (fat/skin for poultry).

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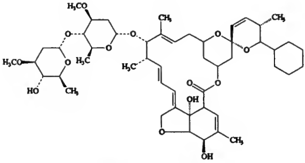
DORAMECTIN

First draft prepard by
Dr. Ray Heitzam
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical name: 25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl)avermectin A_{1a}

Structural formula:



Doramectin

Synonyms: Doramectin, Dectomax®, UK-67,994

Molecular formula: $C_{50}H_{74}O_{14}$

Molecular weight: 899.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Doramectin

Appearance: White to light tan powder

Melting point (average): 160.5°- 162.2°C

Solubility (at 22-25°C): water 0.0003 g/l
acetonitrile 33 g/l
methylene chloride 530 g/l

Optical Rotation: + 12.2° (anhydrous)

UV_{max}: 244 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Doramectin is administered to cattle for the treatment of endo- and ecto-parasitic infections.

Dosage

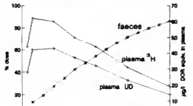
Doramectin is administered to cattle as a single subcutaneous (s.c.) or intramuscular (i.m.) dose of 0.2 mg/kg body weight (b.w.). A repeat dose may be administered eight weeks later.

METABOLISM

Pharmacokinetics

The plasma kinetics of Doramectin were determined in six cattle dosed s.c. with tritiated Doramectin at 0.2 mg/kg b.w. using a prototype commercial formulation (75% sesame oil/25% ethyl oleate) (Pfizer Inc, 1992a). The concentrations of labeled and unlabeled Doramectin and the percentage of the dose excreted in the faeces and urine are shown in Figure 1.

Figure 1. Residues in plasma, faeces and urine of cattle.



Analysis of plasma concentrations used liquid scintillation for quantitation of total residues and chromatographic techniques for the quantitation of unchanged Doramectin (UD).

The apparent terminal half-lives of elimination from plasma of total ^{3}H-labeled materials and unchanged Doramectin were 5.9 and 6.2 days, respectively.

Excretion was almost entirely in the faeces presumably by biliary excretion since significant concentrations of labeled materials were measured in the bile of treated cattle. 86% of the dose was excreted by day 14 and because the curve had not reached a plateau state more drug would be eliminated after day 14. Only about 1% of the label was found in the urine (Pfizer Inc, 1992a).

The drug was well dispersed from the injection site, with less than 1% of the dose remaining at 21 days.

Plasma levels of unchanged Doramectin were also determined in a bioequivalence study comparing s.c. and i.m. administration in cattle. In this study, twenty cattle (10 male castrates and 10 females per group) were treated by either i.m. or s.c. injection with the commercial formulation of the drug at a dose of 0.2 mg/kg b.w. Peak concentration (C_{max}), areas under the concentration curve from 0 to infinity hours post injection ($AUC_{0-\infty}$), and the K_{el} and elimination half-life ($t_{1/2}$) were determined from plasma concentrations. The half-life of elimination from plasma was determined as 8.0 ± 2.9 days and 6.9 ± 1.6 days following s.c. and i.m. administration, respectively. Animals treated by the s.c. route showed a mean $AUC_{0-\infty}$ of 457 ± 66 ng·day/ml (± 1 SD) and a mean C_{max} of 27.8 ± 7.9 ng/ml. Results from the i.m. treatment group showed a mean $AUC_{0-\infty}$ of 475 ± 82 ng·day/ml and a mean C_{max} of 33.1 ± 9.0 ng/ml. The s.c. and i.m. routes of administration were considered to be bioequivalent since the 90% confidence limits on the difference between the mean $AUC_{0-\infty}$ values fell within ± 20% of the mean value for the s.c. group (Pfizer Inc, 1992b).

Metabolism in Food Animals

The biotransformation of Doramectin was investigated in the rat, dog and cattle (1990a). Tissue distribution studies, discussed in detail below, showed that the highest concentrations of total residues were found in liver and fat of treated cattle, with only traces detectable in muscle and kidney. For this reason, metabolite identification studies were limited to liver and fat. The methods employed included gradient liquid chromatography with radiochemical detection and Fast Atom Bombardment mass spectrometry. A tritium label was introduced into the Doramectin molecule at the 5-position with high specificity (minimum 14.1 mCi/mg). The material used ranged in radiopurity from 95 to ≥97.2%. The label was metabolically stable, since less than 1% was recovered as tritiated water from cattle faeces containing 87% of the dose collected over a 14 day withdrawal period (1992a).

In all species, a large portion of the administered dose remained unchanged drug (component UD). The products of Doramectin metabolism were similar in all species investigated. These metabolites were more polar than Doramectin and were the result of O-demethylation in the distal saccharide ring (component C), of hydroxylation of the 24-methyl group (component B) and a combination of these biotransformations (component A).

Table 1 compares the distribution of metabolites in the liver, fat and faeces of cattle, rats and dogs.

Table 1. **Percentage of total radioactivity of the major labeled components in the liver and faeces of cattle, rats and dogs at 2, 3 or 21 days post dose. Rats and dogs were dosed orally with 5 and 3.5 mg/kg b.w. respectively. Cattle received a s.c. injection of 0.2 mg/kg b.w.**

Tissue	Species	Day	% ^{3}H recovered	Component[a]			
				A	B	C	UD
Liver	Cattle	3[b]	95	7	ND	9	70
Liver	Cattle	21[c]	82	8	4.4	6.8	57.8
Liver	Rat		37	2	3	12	18
Liver	Dog		51	ND	ND	12	28
Fat	Cattle	21	74-91	ND	ND	ND	91[f]
Faeces	Cattle		75-82	4	5	14	24
Faeces	Rat		NC[e]	16	14	19	22
Faeces	Dog		46	4	5	8	6

[a] As ^{3}H-5-Doramectin

[b] Study CM-92-01, Pfizer Inc, 1992e

[c] Study CM-93-01, Pfizer Inc, 1993c

[d] ND = Not detected

[e] NC = Not calculated since this sample was used as a reference standard.

[f] 4.8-10.9% (mean 7.4%) of total residues were epi-Doramectin.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Overview

Total drug-related residues were measured by the use of tritium labeled Doramectin. The radiochemical purity of ^{3}H-Doramectin used in these studies was ≥ 97.2%. The ^{3}H-label was found to be metabolically stable since less than 1% of faecal radioactivity was recovered as tritiated water (Pfizer Inc, 1990a, 1992a, 1994d).

Two studies were conducted to determine the depletion of Doramectin residues from cattle tissues (see Table 2).

Table 2. Radiolabeled depletion studies using ^{3}H-Doramectin in cattle.

Dose/route mg/kg b.w.	Number cattle per time point	Sampling times (days)	Tissues	Reference
0.2 s.c.	4 (2 MA, 2 FE)	21, 28, 35	M, L, K, F, I.S.	Pfizer, 1990a 1992a, 1993d
0.2 i.m.	4 (2 MA, 2 FE)	7, 14, 21, 28, 35, 42	M, L, K, F, I.S.	Pfizer, 1994d

MA = male, FE = female, M = muscle, L = liver, K = kidney, F = fat, I.S. = injection site.

The first study, 1535N-60-89-009/010 (Pfizer Inc, 1990a and 1992a), suffered from several limitations that were addressed in a second study, 1535N-60-93-016 (Pfizer Inc, 1994d). These limitations, the preparation of the dosing solution under non-GMP conditions and the use of a less robust analytical method for determination of unchanged drug in tissues, particularly liver, do not render the results of this study invalid. However, a more recent study has been completed using, GMP-quality materials, a wider range of time points and the validated regulatory analytical method for the determination of unchanged drug in tissues.

In the first study, 1535N-60-89-009 (Pfizer Inc, 1990a), calves weighing approximately 175 kg received a single s.c. dose of 0.2 mg/kg b.w. ^{3}H-Doramectin in the commercial prototype formulation. Two calves of each sex were euthanized 21, 28 and 35 days after dose administration and samples of muscle, liver, kidney, fat and injection site collected for analysis of total radioactivity and unchanged Doramectin. Subsequent to the completion of the study, deficiencies were noted in the performance of the HPLC analytical method for the quantitation of Doramectin in liver. The improved assay incorporates a more efficient extraction of residues from liver samples. Re-assay of stored samples using the improved method resulted in quantitation of higher concentrations of Doramectin while reassay using the original method indicated that the residues were stable over the three year storage period. Therefore, samples of liver tissue from this study which had been stored frozen at approximately -20°C for up to three years were re-assayed using the proposed analytical method. The results of this study incorporating the reanalysis data for liver tissue using the improved assay are presented in Table 3 (Pfizer Inc, 1993d). There were no differences between the results for males and females.

Table 3. Radiolabeled residues and unchanged drug (μg/kg) in cattle tissues after a single s.c. dose of 0.2 mg/kg ^{3}H-Doramectin[a].

Tissue	Residue Concentration (μg/kg)[b]	Day 21	Day 28	Day 35
Muscle	Total residues (^{3}H)	5	3	2
	Unchanged drug[c]	2.8	0.9	<2.5
	% Unchanged drug	55	41	NE*
Liver	Total residues	86	48	20
	Unchanged drug[d]	41.4	22	7.2
	% Unchanged drug	48	46	36
Kidney	Total residues	14	9	5
	Unchanged drug	6	2	<2.5
	% Unchanged drug	42	30*	NE*
Fat	Total residues	76	42	22
	Unchanged drug	59	19	<2.5
	% Unchanged drug	75	61*	*
Injection Site	Total residues	328	343	7
	Unchanged Drug	128	353	4*
	% Unchanged drug	32	82	82* (N=1)

[a] Study 1535N-60-89-009/010 (Pfizer Inc, 1990a and 1992a); data are means from four animals at each time.

[b] Expressed as Doramectin equivalents.

[c] For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those unchanged drug concentrations falling below the LOQ. Asterisks (*) indicate that some data were below the LOQ. In such cases, the ratio of unchanged drug to total residues is reported as NE (not estimable) or is based on data from three animals or fewer.

[d] Concentrations of unchanged drug determined in the reanalysis of stored samples using proposed determinative method for Doramectin as reported in AHDM-93-02 (Pfizer Inc, 1993d).

A second total residue depletion study was conducted in cattle. This study used i.m. administration of ^{3}H-Doramectin in the commercial formulation (prepared under GMP conditions) at a single dose of 0.2 mg/kg b.w. Calves with a mean weight of 234 kg (2 per sex) at the time of dosing were euthanized at 7, 14, 21, 28, 35 and 42 days after dosing. Samples of muscle, injection site, kidney, fat and liver were collected for analysis of total radioactivity and unchanged Doramectin using a validated HPLC method. The injection site sampled was processed to provide an indication of the concentration of Doramectin residues at the inner 300 g core and the total 500 g sample (Pfizer Inc, 1994d). The results are presented in Table 4.

The results of both total residue depletion studies clearly indicate that the liver and fat are most appropriate for selection as target tissues since the residue concentrations were the highest and were measurable throughout the 42 day withdrawal period. Figures 2 and 3 show the depletion of total and unchanged Doramectin, respectively, over the 42 day withdrawal period in study 1535N-60-93-016. The depletion of total residues and unchanged Doramectin was found to be nearly linear in all tissues over the 7 to 42 day withdrawal period, with the exception of liver which was linear from days 14 to 42, indicating the distribution to the liver was still occurring at day 7. The depletion of the total residues in the edible tissues is shown in Figure 2.

Table 4. Total radiolabeled residues and unchanged drug concentrations (μg/kg) in cattle tissue after a single i.m. dose of 0.2 mg/kg b.w. [^{3}H]-Doramectin[a]

Tissue	Residue Concentration (μg/kg)[b]	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Muscle	Total Residues	40	20	13	10	< 3[c]	< 3
	Unchanged Drug[c]	33	15.9	10.7	7.78	< 2.35	< 2.13
	% Unchanged	84	78	80	75	NE*	NE*
Liver	Total Residues	470	415	257	120	42	24
	Unchanged drug %	319	253	154	72.2	22.6	13.2
	Unchanged	68	61	60	61	53	56
Kidney	Total Residues	108	60	35	22	7	4
	Unchanged Drug	96.2	52.1	28.1	16.7	< 4.85*	3.11
	% Unchanged	89	87	81	77	71*	77
Fat	Total Residues	551	265	180	115	36	23
	Unchanged Drug	493	230	155	102	25.6	16.7
	% Unchanged	89	87	86	88	74*	73
Injection Site (300 g)	Total Residues	2530	303	206	113	< 32	< 18
	Unchanged Drug	2230	272	174	93.4	< 15.7	12.9
	% Unchanged	90	82	83	84	60*	78
Injection Site (500 g)	Total Residues	2540	672	421	571	< 24	18
	Unchanged drug	2300	594	264	331	< 12.6	16.4
	% Unchanged	89	80	75	59	64*	91*

[a] Study 1535N-60-93-016 (Pfizer Inc, 1994d), data are means from four animals (2/sex) at each time point.

[b] Expressed as Doramectin equivalents.

[c] For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those unchanged drug concentrations falling below the LOQ. Asterisks (*) indicate that some individual sample values were below the LOQ. In such cases, the ratio of unchanged drug to total residues is reported as NE (not estimable) or is based on data from three animals or fewer.

The ratio of unchanged drug to total residues was found to be essentially constant over the 42-day withdrawal period in all tissues. The ratio of unchanged drug in all tissues was found to range from 53 to 91% with an overall mean of 76%, in liver from 68% on day 7 to 53% on day 35, with an overall mean of 60% and in fat from 89% on day 7 to 73% on day 42, with an overall mean of 83% (Pfizer Inc, 1994d). The total residues and the content of unchanged drug for fat and liver are shown graphically in Figure 3.

Figure 2. The depletion of the total residues of ^{3}H-Doramectin in cattle tissues after an i.m. dose of 0.2 mg/kg b.w.

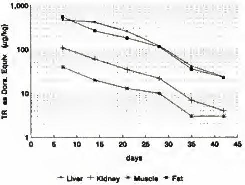
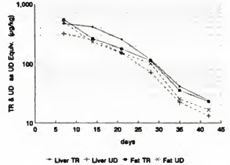


Figure 3. Residues in fat and liver of cattle treated with an i.m. dose of ^{3}H-Doramectin at 0.2 mg/kg b.w.



TR is total residues; UD is unchanged drug

Residues at the Injection Site

Concentrations of total residues and unchanged Doramectin in 300 and 500 g injection site samples showed considerable inter animal variability, an important factor in the observed differences in the means shown in Table 5. However, by day 35 post-dose the concentrations of total residues and unchanged drug in edible tissues were found to be less than 100 µg/kg and essentially similar in injection site samples regardless of the size of the injection site sample.

Other Depletion Studies (with Unlabeled Drug)

Cattle

The depletion of Doramectin from cattle tissues was evaluated in three marker residue depletion studies, two of which were conducted by Pfizer in the US and one in Australia by Commonwealth Scientific and Industrial Research Organization (CSIRO) and McMaster Labs. Two of these studies, 1531N-60-90-049and 2139A-14-92-190 used s.c. administration of Doramectin while the third, 1531N-60-91-050 used i.m. administration of the drug. In all three studies six animals, three per sex, were euthanized at each time point. The results of the three studies are presented in Tables 5, 6 and 7 (Pfizer Inc, 1991a, 1992d, 1994a).

Table 5. **Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single s.c. dose of 0.2 mg/kg b.w. of Doramectin[a]**

Tissue	Day 14	Day 21	Day 28	Day 35
Muscle	13	<7[b]	<4	<3
Liver[c]	177	107	66	29
Kidney	23	11	8.8	<4.5 ± 2
Fat	288	182	94	57
I.S.	7300	1900	380	930

[a] Study 1531N-60-90-049 (Pfizer Inc, 1991a). Data are means from 6 animals (3/sex) at each time point.

[b] LOQ: 2.5 μg/kg for liver, muscle, kidney and injection site, 5 μg/kg for fat. For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those concentrations falling below the LOQ.

[c] Concentrations in liver were obtained using proposed determinative method as reported in AHDM-93-02 (Pfizer Inc, 1993d)

Table 6. **Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single i.m. dose of 0.2 mg/kg b.w. Doramectin[a]**

Tissue	Day 14	Day 21	Day 28	Day 35
Muscle	12	7	3	<2[b]
Liver[c]	116	63	30	27
Kidney	24	12	4	3
Fat	182	97	48	37
Inj. Site	838	1033	162	177

[a] Study 1531N-60-91-050 (Pfizer Inc, 1992d). Data are means from 6 animals (3/sex) at each time point.

[b] LOQ: 2.5 μg/kg for liver, muscle, kidney and injection site, 5 μg/kg for fat. For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those concentrations falling below the LOQ.

[c] Concentrations in liver were obtained using proposed determinative method as reported in AHDM-93-02 (Pfizer Inc, 1993d)

Table 7. **Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single s.c. dose of 0.2 mg/kg b.w. Doramectin[a]**

Tissue	Day 35	Day 42	Day 49	Day 56
Muscle	<2.9[b]	<2.5	<2.5	NM[c]
Liver	15.9	6.6	3.4	NM
Kidney	<3.6	<2.5	<2.6	NM
Fat	16.6	<10.2	<3.9	NM

[a] Study 2539A-14-92-190 (CSIRO study) (Pfizer Inc, 1994d). Data are means from 6 animals (3/sex) at each time point.

[b] LOQ = 2.5 μg/kg. For calculation of the mean 2.5 μg/kg was substituted for those concentrations falling below the LOQ.

[c] Day 56 samples were not analyzed since day 49 samples were generally below the "practical regulatory limit of analytical determination" defined by the authors as 10 μg/kg.

In their monograph (Pfizer 1994e) the sponsors claim that a statistical analysis of the data obtained in the three studies indicated that the slopes of the three depletion curves were parallel. Furthermore, the variances in the data were similar so that it was appropriate to pool the data to estimate a common depletion curve.

In all three studies the residues of parent drug were highest at the injection site, fat and liver and lowest in muscle and kidney. When all the values for the three studies were plotted for liver and fat the values for the regression line reached 100 μg/kg and 10 μg/kg at 19 days and 40 days for liver and 23 days and 43 days for fat (see Figures 4a and 4b). The elimination half lives in liver and fat were about 7.1 days and 6.7 days respectively.

The elimination half-lives and the regression coefficient of both total residues and unchanged Doramectin from liver and fat of cattle medicated with a single parental dose (i.m. or s.c.) of 0.2 mg/kg b.w. Doramectin were calculated from the individual values (Pfizer 1990a, 1993d, 1994d) and are shown in Table 8. The r values suggest some variance between the individual values, nevertheless the elimination half-lives calculated from the semi-logarithmic regression all lie between 5.6 and 8.1 days and there is no significant difference between the values for either the treatment or the total residues and residues of unchanged drug.

Table 8. **Elimination of Doramectin residues from liver and fat after dosing cattle with a parental administration of 0.2 mg/kg b.w. ^{3}H-Doramectin.**

Tissue	Route	Residue	Sampling Period (days)	Half-life (days)	r value
Liver	i.m.	Total	14-42	6.44	-0.942
Liver	i.m.	Doramectin	14-42	6.19	-0.940
Liver	s.c.	Total	21-35	6.71	-0.843
Liver	s.c.	Doramectin	21-35	5.59	-0.872
Fat	i.m.	Total	7-42	7.43	-0.964
Fat	i.m.	Doramectin	7-42	6.92	-0.951
Fat	s.c.	Total	21-35	8.06	-0.823
Fat	s.c.	Doramectin	21-35	7.8	-0.812

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Bound Residues/Bioavailability

The results of the metabolism and total residue depletion studies conducted with [^{3}H]-Doramectin indicate that Doramectin residues are not tightly bound to tissues. Extraction of Doramectin from all edible tissues of cattle treated with the drug results in quantitation of nearly identical levels of total residues, as quantitated by LSC analysis, and unchanged Doramectin, as quantitated by HPLC. Only in liver are levels of unchanged drug and total Doramectin residues appreciably different; however, this difference is related to the presence of a greater proportion of metabolites rather than tighter binding of unchanged Doramectin to the tissue (Pfizer Inc, 1992e, 1993c, 1994d).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Doramectin concentration in cattle tissues was determined by a validated HPLC method. The detection and quantitation of Doramectin at the μg/kg level was based on the extraction from tissue homogenate or fat and subsequent conversion to a chemically stable aromatic fluorescent derivative. The method was specific for Doramectin and showed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin.

High Performance Liquid Chromatography (HPLC)

Liver, kidney, muscle and injection site samples were extracted by incubation at 55°C followed by re-homogenization in the presence of extraction solvent. Fat samples were extracted using incubation at 55°C in hexane followed by homogenization and re-partitioning into acetonitrile. The extracted residues were derivatized using trifluoroacetic anhydride and triethylamine, minimizing exposure to moisture, followed by treatment with methanolic ammonia to yield a fluorescent derivative that was stable to moisture. It was necessary to protect the derivative from light using amber coloured glass vials. Separation was effected using a reverse-phase C18 HPLC-system using acetonitrile, tetrahydrofuran and water in the mobile phase. The amount of Doramectin in each sample was quantitated with or without the use of an internal standard, UK-71,647. The assay showed good sensitivity with a lower LOQ of 2.5 μg/kg and an upper LOQ of 400 μg/kg in liver, kidney, muscle and injection site, and a lower LOQ and upper LOQ of 5 μg/kg and 400 μg/kg, respectively, in fat. Recovery of Doramectin from liver and fat were in excess of 80% and the accuracy of the method was found to be better than 90% and the precision, expressed as the coefficient of variation, was ≤ 10% (Pfizer Inc, 1993a, 1993b, 1994b).

High Performance Liquid Chromatography - Mass Spectrometry (HPLC - MS)

The presence of Doramectin in cattle liver and fat may be confirmed at trace (μg/kg) levels using HPLC - MS techniques. Doramectin was extracted from liver or fat using the same methods employed in the determinative HPLC method. Extracts from both tissues were then subjected to clean up by analyte partitioning and re-partitioning into acetonitrile and hexane or elution from a solid phase extraction column. The extract was then analyzed by HPLC-MS/MS, using a triple quadrupole mass spectrometer equipped with an ion spray HPLC interface. Daughter ions (m/z 145, 331, 593, and 899) of the Doramectin ammonium adduct (m/z 916) generated by collision activated dissociation were monitored. The analyses of tissues fortified with 25 to 250 μg/kg Doramectin were successfully carried out as was the analysis of tissues incurred with Doramectin residues at concentrations predetermined to be in the range of 50 and 85 μg/kg. The method was specific for the confirmatory identification of Doramectin in tissue. No interference was found when ivermectin was analyzed by this method (Pfizer Inc, 1992c, 1994c).

APPRAISAL

The assessment of this parasiticide was made easier by the limitations for its use. The drug is only intended for use in non-lactating cattle. There are two applications, a s.c. or an i.m. injection at a dose of 0.2 mg/kg b.w.

Pharmacokinetics

Doramectin was equally well absorbed from the intramuscular (i.m.) and subcutaneous (s.c.) sites of injection. Most of the dose (>86%) was eliminated by day 14 througb the faeces with <1% eliminated in the urine.

Metabolism

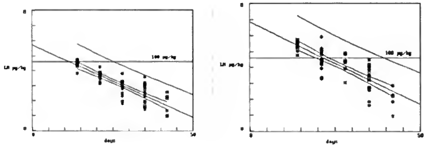
The metabolism was studied in cattle, rat and dog. The biotransformation was similar in the three species. Unchanged drug was the major component of residues in all tissues and its concentration was linearly related to the concentration of total residues in liver and fat. Three minor metabolites were identified in liver, fat and faeces. These metabolites were more polar than Doramectin and were the result of O-demethylation in the distal saccharide ring, of hydroxylation of the 24-methyl group and a combination of these biotransformations. More than 90% of the residues were readily extracted from the tissues.

Residues Depletion

Two residue depletion studies were carried out using ^{3}H-labeled Doramectin at the recommended parenteral dose of 0.2 mg/kg b.w. The concentrations of total residues and unchanged drug were measured in the edible tissues and the injection site. In the first study the dose was administered as a s.c. injection and cattle were sampled between days 21 and 35. In the second study the dosing was an i.m. injection and samples were assayed for the period 7 to 42 days post injection. The bighest levels of residues in the edible tissues were found in fat and liver. The depletion of total residues and unchanged Doramectin was near linear (semi-logarithmic plot) in all tissues over the sampling periods, with the exception of liver in study two which was only linear from days 14 to 42. The total residues at the injection site were approximately 2.5 mg/kg at day 7 and depleted to values below 100 μg/kg by day 35. The rate of depletion was essentially the same for total residues and unchanged drug in liver and fat with elimination half lives varying between 5.6 and 8.1 days in the studies. Combining the results of both studies the mean percentage of unchanged drug in the total residues was approximately 55% in liver and 80% in fat.

Three studies were reported for measuring the depletion of unlabeled drug in cattle after treatment with either a s.c. or an i.m. injection at the recommended dose of 0.2 mg/kg b.w. The concentrations of the residues appeared slightly higher in the tissues of the cattle given the s.c. dose compared to those in the animals injected i.m. The residues of parent drug were highest at the injection site, fat and liver and lowest in muscle and kidney. For example the mean concentrations in μg/kg at 35 days post dosing by the s.c. and the i.m. routes respectively were 930 and 177 for the injection site, 57 and 37 for fat, 29 and 27 for liver, <3 and <2 in muscle and <5 and 3 μg/kg for kidney. The results from the three studies using parental doses of unlabeled Doramectin were combined for fat (Figure 4a) and liver (Figure 4b). The values for tbe regression line reached 100 μg/kg and 10 μg/kg at 19 days and 40 days for liver and 23 days and 43 days for fat respectively.

Figure 4. Residues of Doramectin in fat and liver following parental dosing of 0.2 mg/kg b.w. in cattle.



4a Fat

4b Liver

Fat: $t_{1/2}$ = 6.7 days;
r = -0.824

Liver: $t_{1/2}$ 7.1 days;
r = -0.866

The upper curve is the curve to show the limit for the 99th percentile with a 95% confidence that the values would not be greater. The other curves are the regression line bounded by the 95% confidence limits (CL).

The concentration of total residues at the injection site, as measured on day 35, was much lower using radiolabeled drug (7 µg/kg) compared with the concentrations of parent drug of 930 µg/kg and 177 µg/kg for the s.c. and i.m. samples, respectively, in the unlabeled drug studies. There was clearly great variability in the concentration of residues at the injection site.

A analytical method using HPLC was suitable for monitoring residues of parent drug in the edible tissues and injection site. The method was further modified to include mass spectrometry and this provides a confirmatory method. The detection and quantitation of Doramectin at the µg/kg level was based on the extraction from tissue homogenate or fat and subsequent conversion to a chemically stable aromatic fluorescent derivative. The method was specific for Doramectin and showed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin. The assay showed good sensitivity with an LOQ of 2.5 µg/kg in liver, kidney, muscle and injection site and 5 µg/kg for fat and was linear up to 400 µg/kg. Recovery of Doramectin from liver and fat were in excess of 80% and the accuracy of the method was found to be better than 90% and the precision, expressed as the coefficient of variation, was ≤ 10%.

Doramectin residues were confirmed by analysis using HPLC-MS/MS. The analyses of tissues fortified with 25 to 250 µg/kg Doramectin were successfully carried out as was the analysis of tissues incurred with Doramectin residues at concentrations predetermined to be in the range of 50 and 85 µg/kg. Ivermectin did not cause interference with the confirmation of the method.

Maximum Residue Limits

The ADI of 0-0.5 µg/kg of body weight established by the Committee is equivalent to 30 µg per day for a 60-kg person. In recommending MRLs the Committee took account of the following factors:

- the drug is only intended for use in non-lactating cattle;

- the target tissues are fat and liver;

- the parent drug is the marker residue and that the percentage of residues of parent drug out of the total residues in each tissue are 55% for liver, 80% for fat, 70% for muscle and 75% for kidney;

- there are <10% bound residues;

- no multiple or repeat doses are administered; and

- the LOQs of the analytical methods are 2.5 $\mu g/kg$ for muscle, liver and kidney and 5 $\mu g/kg$ for fat.

The Committee recommends MRLs for cattle of 150 $\mu g/kg$ in fat, 100 $\mu g/kg$ in liver, 10 $\mu g/kg$ in muscle and of 30 $\mu g/kg$ for kidney expressed as parent drug. Using these values for the MRLs then the maximum theoretical intake of residues could be 33 μg, expressed as Doramectin equivalents. This would be compatible with a maximum ADI of 30 μg for a 60-kg person.

	MRL ($\mu g/kg$)	Factor TR/UD	Total Residues ($\mu g/kg$)	Daily food intake (g)	Residue consumed (μg UD equivalents)
Muscle	10	100/70	14	300	4
Liver	100	100/55	182	100	18
Kidney	30	100/75	40	50	2
Fat	150	100/80	188	50	9
Total					33

UD is unchanged parent drug; TR is total residues as UD equivalents.

The sponsors have not submitted data for higher doses than 0.2 mg/kg b.w. whether administered as single doses or as multiple spaced repeat doses. The Committee also notes the high concentrations of residues at the injection sites during the 35 day period after parenteral administration of the unlabeled recommended dose.

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FENBENDAZOLE

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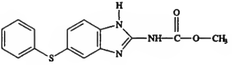
IDENTITY

Fenbendazole is a member of a well-known and widely used chemical class of compounds known as the benzimidazoles. It is related in chemical structure and pharmacological properties to other compounds such as thiabendazole, febantel, oxibendazole, mebendazole, oxfendazole and triclabendazole.

Chemical name:	Methyl 5-(phenylthio)-2-benzimidazolecarbamate

Structural formula:



Fenbendazole

CAS number:	43210-67-9
Synonyms:	PANACUR®, SAFE-GUARD®, HOE 881, AXILUR®
Molecular formula:	$C_{15}H_{13}N_3O_2S$
Molecular weight:	299.35

CONDITIONS OF USE

Fenbendazole is an anthelmintic used for the treatment and control of helminth infections in many mammalian and avian species (e.g. cattle, sheep, goats, horses, pigs, ...). Fenbendazole has a broad spectrum of activity against all stages of gastrointestinal nematodes including larval stages, cestodes and lungworms. It is administered as a single therapeutic dose or over several days in divided doses depending on the species.

Dosage

Typical single therapeutic doses for cattle, sheep, goats, horses and pigs are 5-10, 5-10, 5, 5-10 and 5 mg/kg, respectively.

PREVIOUS REVIEWS

Febantel, fenbendazole and oxfendazole were previously reviewed at the 38th meeting of JECFA.

The Committee recommended temporary MRLs for febantel, fenbendazole and oxfendazole of 100 μg/kg for muscle, fat and kidney (cattle, sheep, and pigs); 500 μg/kg for liver (cattle, sheep, and pigs); and 100 μg/L for milk (cattle). The temporary MRLs are for febantel, fenbendazole, and oxfendazole individually or in combination. The MRL value is the sum of the concentrations of fenbendazole, oxfendazole, and oxfendazole sulfone, calculated as oxfendazole sulfone equivalents.

The Committee requested the results of the following residue studies for evaluation:

1. Studies on the total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone), measured as oxfendazole sulfone, in the edible tissues of cattle and sheep and in the milk of cattle over a 28-day withdrawal period after treatment of animals with fenbendazole or oxfendazole. In particular, information is requested on the use of the pelleted form of fenbendazole in cattle and sheep.

2. Studies on the total residues of the above three metabolites, measured as oxfendazole sulfone, in the edible tissues of pigs given fenbendazole and observed over a 7-14 day withdrawal period.

3. Information on the bioavailability of bound residues in liver after administration of febantel to one of the following species: cattle, pigs, or sheep.

4. Development of a suitable method for the determination of total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone, measure as oxfendazole sulfone) in milk.

1995 DATA SUBMISSION

There were no new data submitted for either febantel or oxfendazole. The residue data submitted for fenbendazole consisted of the following studies:

1. An analytical method titled "Determination of Residues of Fenbendazole (HOE 881, Panacur®) and its Metabolites in Bovine Fatty Tissue, Liver, Kidney and Muscle."

2. Numerous residue depletion studies (including milk) in cattle, sheep, pigs and horses using various formulations, dosing levels and dosing regimes. The studies were designed primarily to determine withdrawal periods for each of the formulations. Some of the depletion studies included the analysis of blood samples in order to compare the pharmacokinetic profile of fenbendazole and its metabolites. The residue studies used an analytical method that converted fenbendazole and its metabolites (oxfendazole and oxfendazole sulfone) to oxfendazole sulfone. The total residue of oxfendazole sulfone was then determined quantitatively, after high pressure liquid chromatography separation with fluorescence detection. The limit of quantitation of the assay in all tissues is 5 μg/kg. One of two milk depletion studies in dairy cattle used both the method that converts the residues to oxfendazole sulfone and an older method using UV-detection with a limit of quantitation of 5-10 μg/l. The UV-detection method measures fenbendazole, oxfendazole and oxfendazole individually. Two milk residue studies in sheep used the summation method.

TISSUE RESIDUE DEPLETION STUDIES

Nonradiometric Residue Depletion Studies

Cattle

Four new studies were reported, two with the 10% suspension, one with the 22% granules and one with the 1.5% pellet formulation. All four used a 7.5 mg/kg b.w. dose of fenbendazole. With the 10% suspension dose, residues were measured at 5, 9, 14, 21 and 28 days in one study and at 7, 11, 15, 18 and 21 days in the second. In both studies, residues in liver tissue were below the temporary MRL by day 9 and 7, respectively, following drug administration (from 19-46 μg/kg at day 9 in the first study, and 150-237 μg/kg at day 7 in the second study). Residues were below the limit of quantitation (LOQ) in all other tissues (less than 5 μg/kg) at these same time points. In the 22% granules formulation study, residues were measured at 5, 9, 14 and 21 days post treatment. At day 9 post treatment, liver residues were 37-86 μg/kg and below the LOQ in all other tissues. In the study using 1.5% pellets, animals were sampled at 7, 14 and 28 days post treatment. At 14 days post treatment, liver residues were 7-15 μg/kg and below the LOQ in all other tissues.

The pharmacokinetics data indicated that there were statistically significant differences in the pharmacokinetics of fenbendazole and oxfendazole with the different formulations. The pelleted formulation was about 400% more bioavailable than the 10% suspension formulation. The data did not permit a determination of whether this has any impact on the depletion patterns of fenbendazole and its metabolites in tissue. Although the pelleted formulation is more bioavailable, the clearance of fenbendazole and metabolites from plasma is similar, based on the time for all samples to reach the LOQ (144-168 hours). The data are summarized in Table 1.

Table 1. Pharmacokinetics Data on 1.5% Pellet Formulation Versus 10% Suspension in Cattle

Formulation	Analyte	C_{max} μg/L	T_{max} (h)	Post Treatment Hours To All Values < LOQ
1.5% Pellets	FBZ	704	32	144
	FBZ-SO	1152	48	168
	$FBZ\text{-}SO_2$	392	80	168
10% Suspension	FBZ	169	24	168
	FBZ-SO	305	24	168
	$FBZ\text{-}SO_2$	134	48	168

FBZ = fenbendazole, FBZ-SO = oxfendazole, $FBZ\text{-}SO_2$ = oxfendazole sulfone

Two studies on total residues in bovine milk were reported, using the 1.5% pelleted formulation and the 10% oral suspension, at a dose of 7.5 mg/kg. In the study using the pelleted formulation, milk samples were collected twice daily until 14 days post treatment. Residues were measured by the older HPLC (LOQ of 5-10 μg/L) and the new summation method (described below). Results from both analyses indicate that total residues were below the temporary MRL (100 μg/kg) by day 5 post treatment. In the second study using the 10% oral suspension administered to 4 high and 4 low yielding cows, the total residue were less than 100 μg/L by 58 and 72 hours, respectively, for the high and low yielding cows. These data also indicate that the 1.5% pelleted formulation has higher bioavailability than the 10% oral suspension formulation.

Sheep

Four tissue residue studies were reported for sheep, two with a 2.5% suspension and two with a 250 mg bolus formulation. Both formulations were evaluated at 5 and 10 mg/kg b.w. The 1.5% pelleted formulation is not approved for use in sheep. In the studies using the 2.5% suspension, blood samples were also collected.

Using a 5 mg/kg b.w. treatment with the 2.5% suspension, blood samples were collected up to 168 h and tissue samples over a 28-day period. Nine days post treatment all tissues except liver were less than 100 μg/kg. Liver residues persisted through day 28, but were below the temporary MRL (500 μg/kg) in all sheep by day 14. At 21 days post treatment liver residues were 10-114 μg/kg and at day 28, residues were 14-19 μg/kg (one animal was below the LOQ). Plasma concentrations were below the LOQ (10 μg/L) by 168 h. The time to reach maximum concentration was 8 h for parent drug, 24 h for the oxfendazole and 48 h for the oxfendazole sulfone metabolites. Maximum concentrations were 137, 226, and 83 μg/L, respectively. Residues of fenbendazole were below the LOQ at 96-120 h; for oxfendazole the corresponding post treatment times were 120-168 h, and for oxfendazole sulfone, low concentrations of this metabolite were still present after 7 days (168 h) in some animals.

In the 10 mg/kg b.w. study using the 2.5% suspension, at 9 days post treatment, muscle, kidney and fat residues were less than 100 μg/kg but liver residues in all sheep were 540-990 μg/kg. At 14 days post treatment, muscle, kidney and fat were below the LOQ and corresponding liver residues were 11-129 μg/kg. By day 21, liver residues were 8-26 μg/kg.

With fenbendazole administered at 5 mg/kg b.w. using the 250 mg bolus (tablet), by day 9, muscle, kidney and fat residues were less than 100 μg/kg and all liver samples were greater than 500 μg/kg. At day 14 two of the four sheep had liver residues of 546 and 532 μg/kg, by 21 days post treatment muscle, kidney and fat residues were below the LOQ and liver residues were 6-65 μg/kg. Plasma residues for fenbendazole and its metabolites are summarized in Table 2. The LOQ for plasma residues was 10 μg/L.

Table 2. Comparative Pharmacokinetics Data on 2.5% Suspension and 250 mg Bolus in Sheep

Formulation	Analyte	C_{max} (μg/L)	T_{max} (h)	Time Post Treatment To Residues < LOQ
2.5% Suspension	FBZ	137	8	120
	FBZ-SO	226	24	168
	FBZ-SO_2	83	48	168
250 mg Bolus (Tablet)	FBZ	242	6	96
	FBZ-SO	302	24	144
	FBZ-SO_2	93	24	168

The pharmacokinetics data indicate that the bolus formulation has approximately 30% more bioavailability than the 2.5% suspension. It was also noted that doubling the dose of the 2.5% suspension fenbendazole formulation resulted in higher liver residues at the day 5 post treatment, but residues were slightly higher at longer post treatment times for the lower dose. It was noted that with the benzimidazoles, both diet and manipulation of feeding can influence the pharmacokinetics of these compounds. However, because of the controlled nature of the studies, inconsistencies in tissue residues between the two dose rates must be a reflection of the variability in absorption and metabolism in sheep following drug treatment.

In the 10 mg/kg treatment using the 250 mg bolus, tissue residues were monitored over a 28 day period post treatment. As in the above studies, at day 9, in muscle, kidney and fat, residues were less than the temporary MRL of 100 μg/kg while all liver samples had residues greater than 500 μg/kg. By day 14 the liver residues in all sheep were 78-471 μg/kg and residues in muscle, kidney and fat were below the LOQ. At day 28, two of the four sheep had liver residues of 11-21 μg/kg.

Two milk residue studies in lactating sheep were reported, one using the 2.5% suspension, the other using the 250 mg bolus at a dose rate of 5 mg/kg b.w. Residues were measured by the new analytical method (HPLC)

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that determines all residues as the oxfendazole sulfone.

In the first study using the 2.5% suspension in eight ewes at 5 mg/kg b.w., residues in all ewes were below 100 µg/L at 24-72 h, and below the LOQ (5 µg/L) at 96-156 h. In the second study using the 250 mg bolus in eight ewes, residues were below the temporary MRL for cattle milk (100 µg/L) at 36-72 h and below the LOQ at 96-132 h.

Pig

Five studies were conducted in pig, one with the 1.5% pellet formulation and the other four with the 4% powder formulation. In two of the studies fenbendazole was given once in the feed at 5 mg/kg b.w. In the remaining three, the drug was administered in feed over several days. Residues were determined using the new analytical procedure and reported as oxfendazole sulfone.

In the first study using the 4% powder formulation at 3 mg/kg b.w. daily for three days, residues were determined at 5, 7, 10 and 14 days. At day 5, one of the six pigs has residues of 10 µg/kg in fat, all muscle, kidney and liver tissue residues were below the LOQ (5 µg/kg). Residues at day 14 in fat were 6-88 µg/kg in four of the six animals.

In two studies, fenbendazole was given as a single dose at 5 mg/kg b.w. using the 1.5% pellet and 4% powder formulation. Residues were determined over a 21 and 27 day post treatment, respectively. Following administration of the 1.5% pellet, residues were detected only sporadically at less than 10 µg/kg in skin, fat, muscle and liver of one or two pigs over the 21-day treatment period. With the 4% powder formulation at 5 mg/kg b.w., residues were detected at only 7 µg/kg once in the skin of one of the five pigs at 5 days post administration. All other tissue residues were below the LOQ at day 5.

In the two remaining studies the 4% powder formulation was used. In one of these studies dosing was 1 mg/kg b.w. per day for 5 days (total dose was 5 mg/kg b.w.). Residues were determined at 5, 7, 10 and 14 days. In the second study, fenbendazole was given at a dose rate of 0.3 mg/kg b.w. per day for 15 days (total dose was 4.5 mg/kg b.w.). Tissue samples were analyzed at 5, 7 and 10 days. Residues were at the LOQ in all tissues (including skin) in all pigs at 5 days.

Horses

As a consequence of horse being considered a minor species as a food animal, only a limited residue study was conducted in four adult horses using the 18.75% paste at a dose rate of 10 mg/kg b.w. (the recommended therapeutic dose in the U.S.). The recommended single dose in Europe is 7.5 mg/kg b.w. Residues were determined at 20 days post treatment and blood samples were collected at regular internals from drug treatment until necropsy. Fenbendazole and its metabolites were detected only during the first 24 hours post treatment. From 48 h until day 20, plasma drug levels were below the LOQ of 10 µg/L. Residues in tissues were below the LOQ (5 µg/kg) in all horses at the 20-day sampling point.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Fenbendazole and its metabolites (oxfendazole and oxfendazole sulfone) can be quantitatively determined in bovine fat, liver, kidney and muscle. Fenbendazole and its metabolites are extracted from tissue homogenates with ethyl acetate. Fenbendazole and oxfendazole are oxidized to oxfendazole sulfone with peracetic acid. The total amount of oxfendazole sulfone is quantitatively analyzed after extensive purification using HPLC with fluorescence detection, 295 nm (Ex.) and 410 nm (Em.). A calibration function is constructed using peak ratios of oxfendazole sulfone and the internal standard determined from tissue samples spiked with various concentrations of a fenbendazole-oxfendazole-oxfendazole sulfone mixture. The recovery of fenbendazole and its metabolites using incurred residues has not been determined, e.g. by using radiolabeled drug. The recovery of oxfendazole sulfone and the internal standard were determined relative to pure aqueous standard solutions. The mean recovery for fenbendazole and its metabolites (measured as $FBZ\text{-}SO_2$) ranged from 70.8% (muscle, 6 samples) to 87.8% (liver, 5 samples). The limit of quantitation for all tissues is 5 µg/kg; the method is linear in the range from 5 to 1000 µg/kg in liver and from 5 to 200 µg/kg in kidney, fat and muscle. The method uses an internal standard, methyl-(5-cyclopentylsulfinyl-1H-benzimidazole-2-yl)carbamate.

APPRAISAL

The Committee recommended that the MRLs for febantel, fenbendazole and oxfendazole should continue be based on the temporary ADI of 0-4 µg per kg of body weight previously allocated to oxfendazole and they should remain temporary. For a 60-kg person, this corresponds to 240 µg/day of residues of febantel, fenbendazole and oxfendazole.

The recommended temporary MRLs for febantel, fenbendazole and oxfendazole, determined as the sum of oxfendazole, fenbendazole and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents, for cattle, sheep and pigs:

Muscle, kidney and fat:	100 µg/kg
Liver:	500 µg/kg
Milk (cattle & sheep):	100 µg/L

These MRLs would results in a maximum daily intake of 240 µg of residues of febantel, fenbendazole and oxfendazole, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat and 1.5 L of milk.

The Committee noted that, with increasing production of goats in developing countries, residue data would be required for establishing MRLs in this species.

The results of ongoing residue depletion studies on total residues of fenbendazole, oxfendazole and oxfendazole sulfone in cattle and sheep following the administration of febantel and oxfendazole are required for evaluation in 1998.

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V-0079-1293-1098.

Schmid, K. (1994). Report on tissue residues of fenbendazole (FBZ) and its metabolites in cattle after oral treatment with Panacur granules ad us. vet. (222 mg fenbendazole/g) at a dose rate of 7.5 mg FBZ/kg body weight. V-0079-0394-1142.

Schmid, K. (1993). Report on plasma concentrations of fenbendazole (FBZ) and its metabolites in bulls after oral treatment with Panacur pellets 1.5% ad us. vet. at a dose rate of 7.5 mg FBZ/kg body weight.
V-0079-0593-1119.

Scholl, T. (1994). Pharmacokinetics and statistics of HOE 881 and its metabolite FBZ-SO in suspension 10% versus pellets 1.5% in cattle. V-0079-0994-1162.

Düwel, (1994). Report on residues in milk and plasma concentrations of fenbendazole (FBZ) and its metabolites in dairy cattle after oral treatment with Panacur pellets 1.5% ad us. vet. at a dose rate of 7.5 mg FBZ/kg body weight. V-0079-0293-1073.

Düwel, (1994). Report on residues in milk and plasma concentrations of fenbendazole (FBZ) and its metabolites in dairy cattle after oral treatment woth Panacur pellets 1.5% ad us. vet. at a dose rate of 7.5 mg FBZ/kg body weight. V-0079-0793-1099.

Schmid, K. (1994). Report on residues of fenbendazole (FBZ) and its metabolites in milk of cattle after oral treatment with Panacur Suispension 10% ad us. vet. at a dose rate of 7.5 mg FBZ/kg body weight. V-0079-0693-1118.

Schmid, K. (1994). Report on plasma concentrations and tissues residues of fenbendazole (FBZ) and its metabolites in sheep after oral treatment with Panacur Suspension 2.5% ad us. vet. at a dose rate of 5.0 mg FBZ/kg body weight. V-0079-0693-1121.

Scholl, T. and Schmid, K. (1993). Report on plasma concentrations and tissue residues of fenbendazole (FBZ) and its metabolites in sheep after oral treatment with Panacur Bolus 250 mg ad us. vet. at a dose rate of 5 mg FBZ/kg body weight. V-0079-1193-1108.

Scholl, T. (1994). Pharmacokinetics and statistics of HOE 881 and its metabolite FBZ-SO in suspension 2.5% versus bolus 250 mg in sheep. V-0079-0894-1150.

Schmid, K. (1994). Report on residues of fenbendazole and its metabolites in sheep after oral treatment with Panacur Suspension 2.5% ad us. vet. at a dose rate of 10 mg FBZ/kg body weight. V-0079-1093-1097.

Schmid, K. (1994). Report on tissue residues of fenbendazole (FBZ) and its metabolites in sheep after oral treatment with Panacur Boli 250 ad us. vet. at a dose rate of 10 mg FBZ/kg body weight. V-0079-1293-1110.

Tiefenbach, B. (1993). Report on the determination of the levels of fenbendazole (FBZ) and it metabolites in the milk of sheep treated orally with the Panacur susp. 2.5% for veterinary use at a dose rate of 5.0 mg fenbendazole/kg body weight. V-0079-0593-1070.

Tiefenbach, B. and Scholl, T. (1993). Report on the determination of the levels of fenbendazole (FBZ) and it metabolites in the milk of sheep treated orally with the Panacur Boli 250 for veterinary use at a dose rate of 5.0 mg fenbendazole/kg body weight. V-0079-0593-1071.

Schmid, K. (1993). Report on plasma concentrations and tissue residues of fenbendazole (FBZ) and its metabolites in pigs after oral treatment with Panacur powder 4% ad us. vet. at a dose rate of 3 mg FBZ/kg body weight daily for 3 days. V-0079-0793-1092.

Scholl, T. and Schmid, K. (1993). Report on plasma concentrations and residues of fenbendazole (FBZ) and its metabolites in pigs after oral treatment with Panacur pellets 1.5% ad us. vet. at a dose rate of 5 mg FBZ/kg b.w. V-0079-0993-1117.

Schmid, K. (1993). Report on tissue residues of fenbendazole (FBZ) and its metabolites in pigs after oral treatment with Panacur powder 4% ad us. vet. at a dose rate of 5 mg FBZ/kg body weight. V-0079-1193-1120.

Schmid, K. (1994). Report on tissue residues of fenbendazole (FBZ) and its metabolites in pigs after oral treatment with Panacur Powder 4% ad us. vet. at a dose rate of 1 mg FBZ/kg body weight/day for 5 days. V-0079-1293-1111.

Schmid, K. (1994). Report on tissue residues of fenbendazole (FBZ) and its metabolites in pigs after oral treatment with fenbendazole at 0.3 mg/kg body weight/day for 15 days (Panacur Powder 4% ad us. vet.). V-0079-0294-1130.

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Scholl, T. and Schmid, K. (1993). Report on plasma levels and tissues residues of fenbendazole and its metabolites in horses after otal treatment with Panacur Paste ad us. vet. at a dose rate of 10 mg FBZ/kg b.w. V-0079-1293-1109.

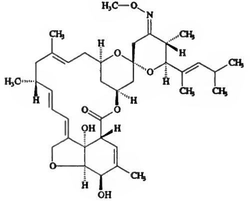
MOXIDECTIN

First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical Name: Spiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]-benzodioxacyclooctadecin-13,2'-[2H]pyran-17-one]-6'-[1,3-dimethyl-1-butenyl]-3',4',5',6,6',7,10,11,14,15,17a,20,20a,20b-dihydro-4'-[methoxyimino]-5',6,8,19-tetramethyl-[6R-[2aE,4E,4'E,5'S*,6R*,6'S*(E),8E,11R*,13R*,15S*,17aR*,20R*,20aR*,20bS*]]-

Structural formula:



Moxidectin

CAS number: 113507-06-5

Molecular formula: $C_{37}H_{53}NO_8$

Molecular weight: 639.84

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White to pale yellow crystalline powder.

Melting point: Glass transition at 110°C, 145-154°C.

Solubility:	0.51 mg/l water; very soluble in polar organic solvents
Optical rotation:	+104 ± 2.7°
UV maxima:	242 nm in acetonitrile
Fluorescence:	Excitation 380 nm, emission 464 nm
Stability:	Can be stored at 4-25°C for 12 months.
	Moxidectin is a very weak base with a $pKa < 2$
Purity of veterinary preparation:	>90% with <9% Moxidectin related minor components

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Moxidectin is used to control a broad range of internal and external parasites in food producing and companion animals.

Dosage

1. As a subcutaneous injection of 0.2 mg/kg body weight (b.w.) to cattle and sheep
2. As an oral drench of 0.2 mg/kg body weight to sheep
3. As a pour-on dosage of 0.5 mg/kg body weight for cattle and deer

METABOLISM

Pharmacokinetics

Radiolabeled Moxidectin

The radiolabeled materials used were either ^{14}C or ^{3}H-labelled Moxidectin. The ^{3}H-Moxidectin was used only for a preliminary study in cattle. ^{14}C-Moxidectin with a radiochemical purity of 94-98% was used in studies on rats, cattle and sheep.

Excretion into Faeces and Urine

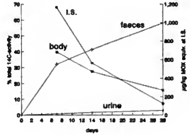
Rats

Male and female rats were administered a single oral dose (1.5 mg/kg b.w. or 12 mg/kg b.w.) or as a daily dose (1.5 mg/kg) repeated for 7 days. The excretion into urine and faeces was measured for 7 days after dosing. Total ^{14}C-recovery averaged 86 to 90% for both sexes and dose levels. The primary route of excretion was through the faeces, 81% for the low dose and 75% for the higher dose. Less than 1% of the radioactivity was excreted via the urine.

Cattle

Three steers averaging 224 kg b.w. were administered a single s.c. injection of ^{14}C-Moxidectin providing 0.2 mg/kg b.w. (MR19). The distribution of the radioactivity is shown in Figure 1.

Figure 1. **Radioactivity accumulated in excreta and remaining in the body of steers after s.c. injection of ^{14}C-Moxidectin at 0.2 mg/kg b.w.**



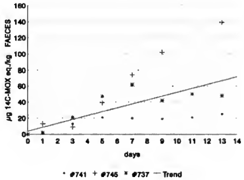
Each time point represents data for a separate steer.

The results for the body include all tissues and body fluids except the head, feet, tail and some large bones which could not be ground. The residues at the injection site (I.S.) are in µg/kg of ^{14}C-Moxidectin equivalents. 96.9 to 98.0% of the residue at the injection site are unchanged drug.

Moxidectin was readily absorbed from the injection site with a half life of about 6.55 days (r = 1.000). In a preliminary study (MR9) using s.c. 0.4 mg/kg b.w. ^{3}H-Moxidectin, the half life at the injection site was 6.4 days. The faeces were the primary route of excretion with <3% of the radioactivity excreted into the urine. This suggests that the large molecule of Moxidectin is not broken down into much smaller molecules which would be cleared into the urine via the kidneys but rather that the molecule and any metabolites are excreted via the bile. Also the residues remaining in the body are clearing gradually indicating that the radioactive residues have a long half life (see below).

Six steers were administered with a pour-on preparation of ^{14}C-Moxidectin at a dose of 0.5 mg/kg b.w. Three steers were slaughtered two days after treatment and three steers at 14 days after treatment. The concentrations of total residues in the urine and faeces were measured daily. The results are shown in Figure 2 for the steers slaughtered at 14 days.

Figure 2. **Excretion of radioactivity as ^{14}C-Moxidectin equivalents into faeces of steers treated with a pour-on preparation of Moxidectin at a dose of 0.5 mg/kg b.w.**



The excretion into the urine was very low compared to that into the faeces. No radioactivity above the limit of detection (2 µg/l) was detected in the urine of the three steers up to slaughter at 2 days post dosing and in the remaining three steers for the period up to 9 days. Thereafter residues were detected in steers # 745 and #737 between days 10 and 14. The concentrations (µg/l) of residues were:

Steer Days after dosing	0-9	10	11	12	13	14
#741	<2	<2	<2	<2	<2	<2
#745	<2	18	11	12	7	5
#737	<2	2	2	<2	2	<2

Blood Pharmacokinetics

Cattle, sheep and rats were dosed with ^{14}C-Moxidectin (see Table 1 for details) and the radioactivity in blood was monitored with respect to time. The percentage of the dose absorbed, the maximum concentration and the time to peak concentration were calculated and the results are shown in Table 1.

Table 1. Pharmacokinetic Parameters (mean ± 1 SD) for Moxidectin in whole blood following oral or subcutaneous administration in rats, cattle and sheep

Parameter Number	Rats[1] (oral) n=9	Sheep[2] (oral) n=2[5]	Sheep[3] (s.c.) n=3	Cattle[4] (s.c.) n=3
Absorption (%)	18.6 ± 4.6	24.4, 21.0	75.9 ± 18.3	103.3 ± 12.0
C_{max} (µg/l)	13.1 ± 2.3	8, 9	12.3 ± 1.2	47.7 ± 9.3
T_{max} (h)	4.8 ± 1.2	10, 8	8.0 ± 2.0	7.3 ± 4.2
$t_{1/2}$(h)	23^{m}, 45^{f}	18, 21	88	75 ± 19
Reference	MR17	MR2	MR2	MR20

[1] Rats (5m, 4f) were administered a single oral dose of 0.2 mg/kg b.w. (m = male, f = female)
[2] Sheep (wethers) were administered a single oral dose of 0.2 mg/kg b.w.
[3] Sheep (wethers) were administered a single s.c. dose of 0.2 mg/kg b.w.
[4] Steers were administered a single s.c. dose of 0.2 mg/kg b.w.
[5] Individual values for two sheep.

Following subcutaneous administration, ^{14}C-Moxidectin was completely absorbed by cattle and slightly less well absorbed (76% of dose) by sheep. The drug was much less absorbed when administered orally to sheep and rats. The maximum concentration in the blood occurs in less than ten hours, however, the elimination half lives are long in cattle and sheep dosed subcutaneously. When the subcutaneous dose of Moxidectin (as ^{3}H-Moxidectin) was doubled for cattle, the elimination half life was even longer (140 h). (MR9). Comparative analysis of the total ^{14}C in the whole blood, serum and clot of cattle demonstrated that essentially all of the radioactivity was associated with the serum fraction.

Metabolism in food animals and rats

The metabolism of Moxidectin was studied in cattle, sheep and rats. The results are shown in Table 2.

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The identity of the codes is:

- 189,056	C-29/C-30 hydroxymethyl	189,021	C-14 hydroxymethyl
- 189,023	C-4 hydroxymethyl	301,310	23-keto

The residues were extracted from both the edible tissues and faeces with mild organic solvents (acetonitrile, methanol) and water. In all cases the majority (86-95%) of the total radiolabeled residues were extracted, indicating that only a low fraction of the residues could be bound residues.

Table 2. **Metabolism of Moxidectin in rats, cattle and sheep expressed as a percentage of the total residues**

Metabolite Tissue	Rat (oral)[1]	Cattle (s.c.)[2]	Cattle (pour-on)[3]	Sheep (drench)[4]
Moxidectin				
M	63.9	50.0	39	92
L	55.9	40.3	39	51
K	37.2	71.1	55	52
F	86.4	76.4	76^{of}, 81^{bf}	91
189,021				
M	1.4	7.7	11	<1
L	7.5	11.7	17	6
K	2.6	5.3	7	4
F	1.0	1.7	2^{of}, 2^{bf}	1
301,310				
M	<0.1	nd	nd	nd
L	0.7	nd	nd	nd
K	<0.1	nd	nd	nd
F	0.15	nd	nd	nd
189,023				
M	4.2	nd	nd	nd
L	7.5	nd	nd	nd
K	2.9	nd	nd	nd
F	6.9	nd	nd	nd
189,056				
M	<0.1	4.9	10	<1
L	<0.1	9.1	11	12
K	<0.1	2.6	5	12
F	<0.1	1.7	2^{of}, 3^{bf}	2

[1] Rats were administered a single oral dose of 1.5 mg/kg b.w. 7 d previously.

[2] Cattle were administered a single subcutaneous dose of 0.2 mg/kg b.w. 14 d previously.

[3] Cattle were administered a single topical application of 0.5 mg/kg b.w. 14 days previously.

[4] Sheep were administered a single oral drench dose of 0.2 mg/kg b.w. 7 days previously.

^{of} is omental fat, ^{bf} is back fat, nd is less than limit of detection.

M = muscle; L = liver; K = kidney; F = fat

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

The studies carried out using ruminants are listed in Table 3.

Table 3. **Studies using radiolabeled Moxidectin.**

Radiolabel	Species	Dose/route (mg/kg b.w.)	Sampling time (days)	Reference
^{3}H-Moxidectin	Cattle	0.4 / s.c.	7, 14, 28, 49	MR9
^{14}C-Moxidectin	Cattle	0.2 / s.c.	7, 14, 28	MR19
^{14}C-Moxidectin	Cattle	0.5 / pour-on	2, 14	MR18
^{14}C-Moxidectin	Sheep	0.4 / s.c.	7, 14, 28, 36	MR14
^{14}C-Moxidectin	Sheep	0.2 / oral drench	1, 7, 28	MR1
^{14}C-Moxidectin	Sheep	0.4 / oral drench	7, 14, 28, 36	MR15

Cattle

The total residues in edible tissues was determined using ^{3}H- or ^{14}C-Moxidectin administered either as a s.c. injection or as a pour-on preparation.

Subcutaneous injection administration

Twelve steers were administered a single s.c. injection of ^{3}H-Moxidectin to provide a dose of approximately 0.4 mg/kg b.w. (twice normal dose). Three animals each were sacrificed at 7, 14, 28 and 49 days after injection and samples of edible tissues and the injection site (I.S.) were analyzed for total tritium residues measured as Moxidectin equivalents (MR19). The results are shown in Table 4.

Table 4. **Total Residues of ^{3}H-Moxidectin (μg Moxidectin eq/kg) in steers administered a single s.c. injection of ^{3}H-Moxidectin to provide a dose of approximately 0.4 mg/kg b.w.**

Tissue	7 days	14 days	28 days	49 days
Muscle	29 ± 4.2	39 ± 8.4	< 10	<4
Liver	148 ± 43.2	97 ± 4.2	47 ± 11.1	17 ± 2.9
Kidney	92 ± 39.5	46 ± 2.3	21 ± 4.4	< 10
Fat (back)	920 ± 403	685 ± 126	359 ± 84.5	182 ± 14.1
Fat (omental)	974 ± 306	778 ± 60.4	350 ± 82.8	181 ± 12.2
Inj. Site	6220 ± 4530	570 ± 182	667 ± 882	34 ± 35.3

Three steers were administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.2 mg/kg b.w. (the recommended dose). One animal each was sacrificed at 7, 14 and 28 days after injection and samples of edible tissues were analyzed for total ^{14}C-residues expressed as Moxidectin equivalents (MR9). The results are shown in Table 5.

Table 5. Total Residues of ^{14}C-Moxidectin (µg Moxidectin eq/kg) in steers administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.2 mg/kg b.w.

Tissue	7 days	14 days	28 days
Muscle (loin)	21	10	4
Liver	109	77	31
Kidney	42	38	13
Fat (back)	495	424	186
Fat (omental)	898	636	275

Pour-on administration

Six steers weighing 163 to 167 kg were administered a single topical dose of ^{14}C-Moxidectin providing 0.5 mg/kg b.w. Three animals each were sacrificed at 2 and 14 days after treatment. Total residues were determined in the edible tissues (MR18). The results are shown in Table 6.

Table 6. Total Residues of ^{14}C-Moxidectin (µg Moxidectin equiv./kg) in steers administered a single topical pour-on treatment of ^{14}C-Moxidectin to provide a dose of 0.5 mg/kg b.w.

Tissue	2 Days	µg/kg	14 Days	µg/kg
	Range (n=3)	Mean	Range (n=3)	Mean
Muscle	<2-<2	<2	<2-3	<3
Liver	2-4	3	5-26	12
Kidney	<2-<2	<2	3-18	8
Fat (back)	<2-7	4	12-129	55
Fat (omental)	7-10	8	33-259	113

The residues were much lower when the pour-on formulation was used compared with s.c. injection treatments. No residue data was provided for the skin or subcutaneous tissues close to the place of application.

Sheep

Subcutaneous injection administration

Four wethers were administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w. (twice the recommended dose). One animal each was sacrificed at 7, 14, 28 and 36 days after injection and samples of edible tissues were analyzed for total ^{14}C-residues measured as Moxidectin equivalents (MR14). The results are shown in Table 7.

Table 7. **Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.**

Tissue	7 days	14 days	28 days	36 days
Muscle (loin)	27	23	< 10	< 10
Liver	118	83	16	12
Kidney	54	24	< 10	< 10
Fat (back)	819	363	44	79
Fat (omental)	934	448	49	87

The residues were highest in fat and lowest in muscle. Why the values in fat were higher on day 36 than day 28 was not clear but similar results were observed when an oral drench was administered (see Table 8).

Oral drench administration

Twelve wethers averaging 34 kg b.w. were administered a single oral dose of ^{14}C-Moxidectin providing 0.4 mg/kg b.w. Three animals were sacrificed at 7, 14, 28 and 36 days after treatment. Total residues were determined in the edible tissues (MR15). The results are shown in Table 8.

Table 8. **Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single oral drench of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.**

Tissue	7 days	14 days	28 days	36 days
Muscle (loin)	12	< 10-11	< 10	< 10
Liver	79	45	< 10-17	23
Kidney	22	18	< 10	< 10
Fat (back)	345	284	62[a]	171[a]
Fat (omental)	411	351	70[a]	183[a]

[a] These tissues were reassayed and the high results at day 36 were confirmed.

The residues were highest in fat and lowest in muscle. Why the values in fat were higher on day 36 than day 28 was not clear but similar results were observed when a subcutaneous dose was administered (see Table 7).

In another study eight wethers averaging 36 kg b.w. were administered a single oral dose of ^{14}C-Moxidectin providing 0.2 mg/kg b.w. Three animals were sacrificed at 1 and 7 days and two animals at 28 days after treatment. Total residues were determined in the edible tissues and blood (MR1). The results are shown in Table 9.

The residues were highest in fat. Residues were detected in the blood on day 1 but radioactivity was not measurable by day 7. This might suggest that the drug was rapidly absorbed from the gut.

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Table 9. Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single oral drench of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.

Tissue	1 day n=3	7 days n=3	28 days n=2
Muscle (loin)	25	12	< 4
Liver	135	50	17
Kidney	41	22	< 4
Fat (back)	220	287	113
Fat (omental)	277	322	123
Blood	5	< 2	< 2

Elimination half-lives for cattle and sheep

The half lives were calculated for each tissue using simple semi-logarithmic linear regression analysis of data recorded for residues found at day 7 and later. The results are shown in Table 10 for both cattle and sheep for all the formulations.

Table 10. Half-lives (days) and regression coefficients of total residues in cattle and sheep with various formulations of Moxidectin

Tissue Dose	Cattle s.c. 0.2 mg/kg	Cattle s.c. 0.4 mg/kg	Sheep s.c. 0.4 mg/kg	Sheep drench 0.4 mg/kg
	$t_{1/2}$ r (days)	$t_{1/2}$ r (days)	$t_{1/2}$ r (days)	$t_{1/2}$ r (days)
Muscle	9.0 -0.992	N/A	N/A	N/A
Liver	11.4 -1.000	13.7 -0.978	8.1 -0.984	N/A
Kidney	11.8 -0.967	10.5 -0.917	N/A	N/A
Fat (back)	14.3 -0.983	18.4 -0.943	7.4 -0.909	17.5 -0.677 8.1[a] -0.974
Fat (omental)	12.2 -0.999	17.0 -0.963	7.3 -0.912	15.8 -0.717 7.8[a] -0.968
Inj. Site		6.4 -0.854		
Reference	MR19	MR9	MR14	MR15

[a] The values were calculated after omitting the results for day 36. However the results for day 36 which were much higher values than for day 28 were confirmed after reanalysis (MR15).
N/A indicates that the concentration of the residues were not quantifiable at a sufficient number of time points, usually because they were not detectable at later times.

Ratio of total residues to unchanged drug

Unchanged Moxidectin was the major residue in all edible tissues. The ratio of unchanged drug (parent compound) to total residues was calculated for some of the radiolabeled studies and the results are shown in Table 11.

Table 11. Percentage of total residues as unchanged parent drug in ruminant tissues.

Species Dose (mg/kg b.w.)	Days	Muscle	Liver	Kidney	Back Fat	Omental Fat
Cattle						
s.c. 0.2 mg/kg	7 14 28	62 50 50	48 40 36	74 71 77	83 76 86	95 88 91
pour-on 0.5 mg/kg	14	39	39	55	76	81
Sheep oral 0.2 mg/kg	7	92	51	52	91	
Mean percentage		59	43	66	82	87

Main points from Radiolabeled studies

The residue patterns were similar in cattle and sheep.

1. The residues were lowest when the drug was administered as a pour-on preparation.

2. The residues were always highest in fat with little difference between the residues in omental and back fat.

3. Parent drug was always the major component of the residues, representing about 40-70% of the total residues in muscle, liver and kidney and 75-95% in fat.

4. The residues persisted in fat tissues and the percentage of unchanged drug in the total residues did not alter significantly with time.

5. Fat is recommended as the marker tissue and unchanged drug as the marker compound for all tissues.

6. There is only limited information on residues at injection sites or the tissue in the immediate vicinity of the pour-on application.

Other Residue Depletion Studies (with unlabelled drug)

The list of studies submitted for evaluation are shown in Table 12.

Table 12. Residue studies using unlabelled Moxidectin

Species Tissue	Dose (mg/kg b.w.)	Route	Sampling time (days)	Reference
CATTLE				
M,L,K,F,I.S.	0.2	s.c.	14, 21, 28, 35, 42, 49	MR12
I.S.	2 x 0.2	s.c.	28	MR11
M,L,K,F	0.5	pour-on	7, 14, 21, 28, 35	MR5
M,L,K,F	0.5	pour-on	14, 21, 28, 35, 42	MR8
milk	0.2	s.c.	daily 1-38	MR13
milk	0.2	s.c. 14-70 days prepartum	daily 2-7 postpartum	MR7
SHEEP				
M,L,K,F	0.2	oral drench	7, 14, 20, 28	MR4
M,L,K,F	0.2	oral drench	14, 21, 28, 35, 42	MR3
Fat	0.2 then 0.2 at day 10	s.c. s.c.	10 20, 30, 40, 50	MR21
DEER				
M,L,K,F	0.5	pour-on	7, 14, 21, 28	MR6

M = muscle; L = liver; K = kidney; F = fat; I.S. = injection site

The concentration of residues of the unchanged parent drug, Moxidectin, were determined by an HPLC method. The limit of quantitation (LOQ) was 10 µg/kg tissue and the limit of detection (LOD) 4 µg/kg tissue. In all of the studies residues of Moxidectin in muscle tissue distant from the injection site were below the LOQ. Residues of Moxidectin in liver and kidney were < 10 µg/kg in the studies where either the oral drench or pour-on preparations were used. In the studies using the s.c. dosing, residues were sometimes above the LOQ in liver and kidney during the first month after treatment, however they rarely exceeded 50 µg/kg.

Cattle

Subcutaneous administration

Eighteen steers and eighteen heifers weighing 191-298 kg received a single s.c. injection of 0.2 mg/kg b.w. The animals were sacrificed in groups of six at 7 day intervals. The residues were determined in the edible tissues and at the injection site. The results are shown in Table 13. The 99% upper confidence limits (CL) for the results for fat also were calculated and are shown in Table 13.

Table 13. **Residues of Moxidectin ($\mu g/kg$) in cattle after s.c. injection of 0.2 mg/kg b.w.**

Withdrawal time	Injection Site	Liver	Kidney	Back Fat	99% upper CL for fat
Control	< 10	< 10	< 10	< 10	
14	3269	14	27	275	438
21	3848	15	29	243	402
28	4019	< 10	22	225	367
35	2332	< 10	19	153	332
42	1326	< 10	<10	77	296
49	1178	<10	11	141	261

Pour-on application

Two studies were carried out using a pour-on application of Moxidectin at a dose of 0.5 mg/kg b.w. (MR5, MR8). The animals were sacrificed in groups at 7 day intervals over either a 35 or a 42 day period. The residues were determined in the edible tissues and the results for residues in fat are shown in Table 14. The 99% upper confidence limits (CL) for the results for fat also were calculated and are shown in Table 14. The residues in muscle, liver and kidney were less than the LOQ of 10 $\mu g/kg$ except in one liver sample at 7 days when the concentration was 11 $\mu g/kg$.

Table 14. **Residues ($\mu g/kg$) in bovine fat after administration of a pour-on dose of 0.5 mg/kg b.w.**

Withdrawal time (days)	Australian study (MR5)		USA study (MR8)	
	Mean	upper 99% CL	Mean	upper 99% CL
7	21	70	N/A	N/A
14	36	67	92	201
21	31	63	106	192
28	10	59	77	183
35	< 10		65	174
42			67	165

N/A = not applicable

Milk

Moxidectin, as with other abermectins, would not normally be recommended for use in lactating animals. Nevertheless studies of residues in milk were submitted (MR7, MR13). In the first study four lactating cows were administered a s.c. injection of 0.2 mg/kg b.w. and residues monitored in the milk for the following 25 days. The concentration of Moxidectin was at a maximum on day 1 (range 60-201 $\mu g/kg$) and declined to <20 $\mu g/kg$ by day 14 and were not detectable (LOQ 10 $\mu g/kg$) after day 23.

The second study used thirty three non-lactating late pregnant cows which were given a s.c. dose of 0.2 mg/kg b.w. of Moxidectin at differing times, 1 to 67 days, before parturition. The residues in the milk for the first 7 days after calving were measured. The residues in milk on days 2, 3 and 4 were similar and significantly higher than those on days 5, 6 and 7. The latter values were close to the LOQ of 10 $\mu g/kg$ or below it. The

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residues in the former samples were used to calculate the upper 99% CL for residues in milk with respect to the time of dosing. The 99% CL were:

Time dosed before parturition (days)	14	21	28	35	42	49	56	63	70
Upper 99% CL conc (µg/kg) in milk 2-4 days post-partum	32	30	27	24	21	19	16	13	10

The calves were sacrificed within 24 hours of birth and the residues measured in the edible tissues. No residues were detected in muscle, liver and kidney but were present in fat. Statistical analysis of the data for fat indicated that the 99% upper CL ranged from 122 µg/kg for calves born from cows treated within 14 days of calving to 62 µg/kg for cows receiving Moxidectin 70 days prior to calving.

Sheep

Oral drench administration

Two studies were carried out administering Moxidectin as an oral drench at a dose of 0.2 mg/kg b.w. (MR3, MR4). The animals were sacrificed in groups at 7 day intervals over either a 35 or a 42 day period. The residues were determined in the edible tissues and the results for residues in fat are shown in Table 15. No residues were detected in muscle, liver or kidney.

Table 15. **Residues (µg/kg) in sheep fat after administration of an oral drench dose of 0.2 mg/kg b.w.**

Withdrawal time (days)	Australian study (MR4)		USA study (MR3)
	Omental fat Mean	upper 99% CL	Back fat range
7	66	131	N/A
14	80	116	25 - 58
21	44	104	<10 - 23
28	29	88	<10 - 26
35	N/A		<10
42	N/A		<10

N/A = not applicable

Subcutaneous administration

Thirty lambs received a subcutaneous dose of 0.2 mg/kg b.w. After 10 days six sheep were sacrificed and the remainder received a second injection at the same dose of 0.2 mg/kg b.w. (MR21). The lambs were sacrificed in groups of six at 10 day intervals and the residues of Moxidectin in back fat samples were measured (see Table 16). No residues were measured at the sites of injection or in the other edible tissues. Control lambs were also monitored and no residues were detected.

Table 16. **Residues (μg/kg) of Moxidectin in back fat of sheep receiving one or two subcutaneous doses of Moxidectin at 0.2 mg/kg b.w. The second dose was given 10 days after the first dose.**

Withdrawal time (days)		Mean	Range
1st	2nd		
10	N/A	222	167 - 314
	10	324	197 - 433
	20	234	183 - 284
	30	139	91 - 223
	40	164	91 - 290

Statistical analysis of the above data indicated that the 99% upper CL on residues in fat were 527, 487, 446 and 406 μg/kg at 7, 14, 21 and 28 days after the second injection, respectively.

Deer

Twenty red deer, 15-16 months old, were treated with Moxidectin pour-on at a dose of 0.5 mg/kg b.w. (MR6). Five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the Moxidectin content assayed. All residues were below the LOQ in muscle (<24 μg/kg), liver (<6 μg/kg) and kidney (<11 μg/kg). The residues in fat are shown in Table 17.

Table 17. **Residues (μg/kg) in fat of Red Deer after administration of a pour-on dose of 0.5 mg/kg b.w.**

Withdrawal time (days)	Mean conc in fat	Calculated 99% upper CL
7	126	266
14	155	226
21	57	185
28	31	144

Residues at the Injection Site

The residues at the site of subcutaneous injection were measured using ^{3}H-Moxidectin (MR9), ^{14}C-Moxidectin (MR19) and unlabelled Moxidectin (MR12). The samples of injection site were either by size, 15 cm diameter x 2.5 cm depth or by weight about 500 g. The results were very different between animals and also showed relatively low concentrations in the radiolabeled study and high concentrations in the unlabelled Moxidectin study even though the dose of unlabelled drug was a half that of the labeled dose. A further study was carried out using unlabelled Moxidectin at the same dose, 0.2 mg/kg b.w. by s.c. injection at two separate sites, with the purpose of checking the residues at the injection sites at 28 days after injection. About 500 g of tissue was sampled from each site and after separation the amount of muscle tissue was 433-477 g and the 21-66 g were subcutaneous tissue (MR11). The results for each of the three studies are shown in Table 18.

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Table 18. Residues (µg/kg) of Moxidectin (MOX) at the injection site in steers.

Withdrawal time (days)	^{14}C-MOX (0.2 mg/kg b.w.) Total residues n=1	^{3}H-MOX (0.4 mg/kg b.w.) Total residues n = 3		Unlabelled MOX (0.2 mg/kg b.w.) n = 6 Moxidectin	
		Range	Mean	Range	Mean
7	1168	517-8685	6220		
14	563	359-712	570	932-8664	3269
21	nm	nm		1479-11988	3848
28	127	90-1484	667	821-11376 19-292[m] 2723-11770[n]	4019 99 6709
35	nm	nm		206-9042	2332
42	nm	nm		78-7020	1326
49	nm	<10-79	34	234-3190	1178

[m] and [n] are the results from study MR11.

There was a wide variation in the results between animals. High concentrations of residues were present in samples throughout the withdrawal period after treatment with the recommended dose of the unlabelled preparation. The majority of the residues may be assumed to be in the fatty tissue of the samples. No explanation was offered as to why the total residues in the radiolabeled study using a two times higher dose should be lower than those for parent drug residues in the unlabelled study. Perhaps there was extensive tritium exchange although this will remain an enigma since no measurements were made of the Moxidectin concentration using the HPLC analytical method.

Bound Residues/Bioavailability

The majority of the radiolabeled residues were extractable with mild solvents. The amount of bound residues is small and insufficient to be taken into account in the calculation of MRLs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A practical HPLC with fluorescence detection was developed for the determination of the unchanged drug, Moxidectin, in the edible tissues of sheep and cattle (MR26). Samples were extracted with acetonitrile. The extract was partitioned into hexane and concentrated. The compound was reacted with acetic anhydride, 1-methylimidazole and dimethylformamide to form a fluorescent derivative. Further clean-up was done on a fluorosil Sep-Pak column and the hexane fraction was dried and redissolved in methanol. An aliquot of the methanol mixture was applied to an HPLC column (Zorbax ODS 5 µm, 25 cm x 4.6 mm ID) using aqueous methanol as the eluent. The retention time was approximately 8.6 min. A standard curve was prepared and the concentration of Moxidectin determined by interpolation. The LOQ was 10 µg/kg and the limit of detection was 1-4 µg/kg. The average recoveries were measured at three different laboratories and were 96 ± 13%, 94 ± 14% and 96 ± 16%. The method applied to fat was specific for Moxidectin at the 250 µg/kg level in the presence of several of the most commonly used animal health products, namely, Ivermectin, Monensin, Levamisole, Oxytetracycline, Procaine Penicillin G, Rafoxanide, Sulphamethazine and Trenbolone, at fortified levels of 50 - 200 µg/kg and 20 mg/kg for Fenbendazole (MR29). No other abermectins were tested. A similar method was also applied to deer tissues (MR6). This method used ivermectin as the internal standard, recoveries were 70-95%, LOD and LOQ were 1 and 2 µg/kg respectively. An LC-MS method was described for the confirmation of Moxidectin at the 250 μg/kg level in fat including other macrocyclic lactones (MR31).

APPRAISAL

Pharmacokinetics

^{14}C-Moxidectin was completely absorbed, with peak blood concentrations at 7 - 8 hours, by cattle and slightly less well absorbed (76% of dose) by sheep following sucutaneous administration. When administered orally to sheep and rats the drug was about 20% absorbed. The maximum concentration in the blood occurs in less than ten hours and the half life of Moxidectin is about 80 hours in cattle and sheep.

The excretion of Moxidectin was determined in cattle and rats using radiolabeled drug. The faeces were the primary route of excretion with $<3\%$ in cattle and $<1\%$ in rats of the radioactivity excreted into the urine.

Metabolism

The metabolism of Moxidectin was similar for cattle and sheep where the principle component of the residues was parent drug, accounting for 75-95% of the residues in fat and 40-90% in other edible tissues at 7 to 28 days after dosing cattle and at 7 days after dosing sheep. In rats parent drug was also the major residue. Two minor residues were found in the ruminants and there was one similar minor metabolite in rats and two different minor metabolites. In all studies the majority (86-95%) of the total radiolabeled residues were extracted, indicating that only a low fraction of the residues was bound.

Residues

The total residues were determined in the edible tissues using radiolabeled drug at one or two times the recommended doses for the s.c. and pour-on preparations for cattle and using the s.c. or oral drench preparations for sheep. The main points from the radiolabeled studies were;

1. The depletion of residues were similar in cattle and sheep and independent of the route of administration or formulation.

2. The residues were lowest when the drug was administered as a pour-on preparation.

3. The residues were always highest in fat and lowest in muscle (for example, in steers given a s.c. dose of 0.2 mg/kg b.w. the levels in fat were 424 and 186 μg/kg compared with 10 and 4 μg/kg in muscle for 14 and 28 days respectively). There was little difference between the concentrations of residues in omental and back fat.

Fat and liver are recommended as the target tissues and unchanged drug as the marker compound for all tissues.

Ten residue studies were carried out using the commercial preparations of Moxidectin. Cattle and sheep were treated with the s.c. preparation, cattle and deer with the pour-on preparation and sheep with the oral drench. The residues of unchanged Moxidectin, at time periods ranging from 7 -50 days, were measured using an HPLC method in the edible tissues and milk. In all cases the residues were low, usually <50 μg/kg, in muscle, liver, kidney and milk and much higher in omental and back fat. The sponsors calculated the upper 99% confidence limits (CL) for the concentration of Moxidectin in fat with respect to time. Over a 14 to 28 day withdrawal period the upper 99% CL were in the range 88-438 μg/kg and declined slowly.

The residues at the site of subcutaneous injection were measured using ^{3}H-Moxidectin and unlabelled Moxidectin. The samples of injection site were either by size, 15 cm diameter x 2.5 cm depth, for the radiolabeled treatment or by weight about 500 g in the unlabelled drug studies. The results were very different between animals and also showed relatively low concentrations ($<$ 10-79 μg/kg at 49 days) in the radiolabeled study and high concentrations (e.g. 234-3190 μg/kg at 49 days) in the unlabelled Moxidectin study even though the dose of unlabelled drug was a half that of the labeled dose. No studies were made to measure the residues in the tissue in the immediate vicinity of the pour-on application.

Analytical Methods

An HPLC method with fluorescence detection was developed for the determination of Moxidectin in the edible tissues of sheep and cattle. Samples were extracted with organic solvents. The Moxidectin residues were derivatised and after further clean-up on small disposable chromatography columns, quantitated on an HPLC column. The limit of quantitation (LOQ) was 10 μg/kg and the limit of detection (LOD) was 1-4 μg/kg. The average recoveries were measured at three different laboratories and were 94-96%. The method applied to fat was specific for Moxidectin at the 250 μg/kg level in the presence of several of the most commonly used animal health products, namely, Ivermectin, Monensin, Levamisole, Oxytetracycline, Procaine Penicillin G, Rafoxanide, Sulphamethazine and Trenbolone, at fortified levels of 50-200 μg/kg and 20 mg/kg for Fenbendazole. A similar method was also applied to deer tissues. Ivermectin was used as the internal standard, recoveries were 70-95% and the LOQ was 2 μg/kg. An LC-MS method was described for the confirmation of Moxidectin at the 250 μg/kg level in fat.

Maximum Residue Limits

In reaching its decision on MRLs the Committee took into account the following factors:

- the ADI 0-2 μg/kg of body weight is equivalent to 120 μg per day for a 60-kg person
- fat and liver are the target tissues
- the marker compound is parent drug
- 40% of the total residues in muscle, liver and kidney are unchanged drug and 75% of the total residues in fat are unchanged drug
- bound residues are 5-15% of the total residues and information is not available to discard them from the calculation of MRL
- the LOQ of the analytical method is 10 μg/kg
- the sponsors are not proposing to make the drug available for use in lactating cows and late pregnant cows. Thus residues in milk will not have to be taken into account.

The Committee recommends MRLs for cattle, sheep and deer of 500 μg/kg in fat, 100 μg/kg in liver, 20 μg/kg in muscle and of 50 μg/kg for kidney, expressed as parent drug. Using these values for the MRLs then the maximum theoretical intake of residues could be 79 μg:

	MRL (μg/kg)	Factor TR/UD	Total residues (μg/kg)	Daily Food Intake (g)	Residue consumed (μg UD equivalents)
Muscle	20	100/40	50	300	15
Liver	100	100/40	250	100	25
Kidney	50	100/40	125	50	6
Fat	500	100/75	665	50	33
Total					79

UD is unchanged parent drug; TR is total residues as UD equivalents.

The above MRLs would be compatible with a maximum ADI of 120 μg/person/day.

The Committee recommends a temporary MRL for deer until further information on the marker compound for deer tissues is available. This information was requested for review in 1998.

The Committee noted the very high concentrations and the great variation of residues at the injection site over a 49-day period after dosing cattle.

REFERENCES

All of the references are confidential documents submitted by the sponsors. They are listed in the text by their MR number assigned by the sponsor.

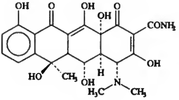
OXYTETRACYCLINE

First draft prepared by
Dr. P. Sinhaseni Tantiyaswasdikul
Department of Pharmacology
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Bangkok, Thailand

IDENTITY

Chemical name: 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Structural formula:



Oxytetracycline

Molecular formula: $C_{22}H_{24}N_2O_9$

Molecular weight: 460.44

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Oxytetracycline is a highly active, broad-spectrum antibiotic which is produced by a fermentation process involving the actinomycete, *Streptomyces rimosus*. Oxytetracycline is present as either the amphoteric base compound, the hydrochloride salt or as a quaternary ammonium salt complex.

The solubility of the base salt varies widely with pH. At 23°it has a minimum solubility of 500 µg/ml at pH 5, 31.4 mg/ml at pH 1.2 and 38.0 mg/ml at pH 9.0. Oxytetracycline crystals show no loss in potency on heating for 4 days at 100°. The amphoteric base forms salts with acids and bases.

The hydrochloride salt is the most common form in parenteral and water soluble animal health products. It is yellow crystalline compound that is odourless and slightly bitter in taste. It is very soluble in water (1 g/ml maximum solubility) and organic solvents. In pure state hydrochloride crystals show $< 5\%$ inactivation after 4 months storage at 56°. Aqueous solutions of the hydrochloride salt at pH 1.0 to 2.5 are stable for at least 30 days at 25° and solutions held at pH 3.0 to 9.0 show no detectable loss in storage at 5° for 30 days.

The quaternary ammonium salt complex is the most common form in feed premixes. Preparation of this complex results from a reaction of the fermentation broth containing oxytetracycline with a monotallow trimethylammonium chloride. This reaction facilitates removal of oxytetracycline from the fermentation broth, and the resulting complex, which is relatively water insoluble, typically contains 50% of oxytetracycline hydrochloride's activity and not more than 15% of the quaternary ammonium compound with remaining components being made up of ash, fermentation solubles and moisture. Feed premix products are mixed to their desired potency by adding carriers such as mineral oil, calcium carbonate, roughage by-products and other components to attain the desired characteristics.

The stability of oxytetracycline in feed premixes has been extensively evaluated and is well established at more than 90% of potency being retained after a 24-month storage under ambient conditions. Other oral dosage forms (tablets and water soluble powders) retain more than 90% of their original potency under ambient conditions for at least 24 and 48 months, respectively. Injectable oxytetracycline products are also very stable as shown by the retention of more than 90% of the potency at least for 24-month storage period.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Oxytetracycline is a broad spectrum antibiotic with considerable activity against gram negative bacteria. It is used in the treatment and control of infections diseases caused by bacteria in giant prawn i.e. vibriosis. It is generally given to prawn by mixing with prepared diets, more often as formulated medicated diet. Oxytetracycline is given to prawn at the concentration range 2-5 g/kg diet *ad libitum* for 5 days. The amount fed is adjusted upon the amount eaten so that prawn always have food available.

METABOLISM AND PHARMACOKINETICS

Metabolism studies

Oxytetracycline metabolism studies had been evaluated at the 36th meeting of JEFCA in several species. The conclusion was the following: "All available evidence suggests minimal , if any, metabolism of the tetracycline antibiotics in rats, dogs or man. Although no useful oxytetracycline metabolism papers have been published, the data available for other tetracyclines allows the conclusion to be reached that no metabolism occurs in animals. Hence a microbiological assay would be expected to detect all residues of oxytetracycline in tissues from animals.".

Pharmacokinetics

The pharmacokinetics of oxytetracycline, absorption, clearance, tissue distribution and excretion, were determined in giant prawn *(Penaeus monodon)* after a single intramuscular injection and forced oral administration at 11 and 22 mg/kg of body weight at 28-30°C. The drug concentrations were analyzed in hemolymph and edible tissues using a microbiological assay and an HPLC method. Hemolymph half-life of 15.5 h was found for 11 mg/kg bw dose admistered intramuscularly. The peak tissue concentrations were found at 8 hours. Tissue half-lives of 19.9 and 18.6 h were found for 11 and 22 mg/kg bw dose levels, respectively. About 78% of the dose was excreted from the hemolymph and 82% from the tissues at 48 h post dosing (Limpoka et al., 1993).

According to the results following intramuscular injection, about 4-fold concentrations of oxytetracycline (OTC) were found in tissues compared with those found in the hemolymph (Table 1). Due to the very low concentrations of OTC found in tissues after oral administration, the drug would not be expected to be detectable in hemolymph (Table 2).

Table 1. Mean prawn tissue and hemolymph concentrations of oxytetracycline (OTC) following a single intramuscular injection at 11 and 22 mg/kg body weight

Time (h)/ Dose	Hemolymph concentration (μg/ml)		Prawn tissue concentration (μg/g)			
	11 mg/kg	22 mg/kg	11 mg/kg		22 mg/kg	
0	0	0	0	0	0	0
0.5	*1.50	*4.0	**8.20±0.95	***6.95±0.02	**20.0±0.85	***11.50±0.6
1	1.30	3.20	8.10±3.0	6.83±0.05	17.50±1.40	11.28±0.90
2	1.15	3.0	7.60±1.45	6.60±0.20	15.0±0.58	10.87±0.84
4	1.10	2.50	7.0±2.0	6.16±0	13.40±0.48	10.09±0.90
8	1.0	2.0	6.50±2.0	5.35±0	10.50±0.62	8.69±0.24
12	0.84	1.95	5.50±2.0	4.66±0.30	8.0±0.18	7.49±0.04
24	0.50	1.65	5.0±0.90	3.06±0.34	7.5±0.62	4.79±0
30	0.40	0.87	5.0±0.15	2.49±0	6.75±0.80	3.83±0.11
48	0.20	0.43	3.20±0.50	1.33±0.01	4.85±0.32	1.96±0.14
54	ND	0.20	2.70±0.75	0.60±0	3.0±0	1.96±0
72	ND	<0.20	2.0±0.85	0.60±0.07	1.70±0	1.28±0.08
96	ND	ND	<1.0	0.20±0	1.70±0	0.20±0
120	ND	ND	ND	0.10±0	<1.0	0.10±0
144	ND	ND	ND	ND	ND	ND
168	ND	ND	ND	ND	ND	ND
192	ND	ND	ND	ND	ND	ND
216	ND	ND	ND	ND	ND	ND
240	ND	ND	ND	ND	ND	ND

* Assay by microbiological method, limit of detection for hemolymph = 0.2 μg/ml
** Assay by microbiological method, limit of detection for prawn tissue = 1.0 μg/g
*** Assay by HPLC, limit of detection = 0.01 μg/g
ND = Not Detected

TISSUE RESIDUE DEPLETION STUDIES

In a residue depletion study 250 prawns, weighing 30-40 g, were assigned to an individual treatment in experimental 2x5 m concrete ponds. Pellet and fish flesh diets, containing each 2.5 and 5.0 g/kg diet of oxytetracycline, were given *ad libitum* for 5 days. Six prawns were collected twice daily for 20 days and analysed by an HPLC method with a detection limit of 0.01 mg/kg. Oxytetracycline concentrations were found to be 3-17 mg/kg and 12-40 mg/kg in muscle tissues of prawns receiving 2.5 g/kg and 5 g/kg of OTC in medicated fish flesh diets for 5 days, respectively, as compared with 0.2-1.5 mg/kg and 1-3 mg/kg, respectively, when the medicated pelleted diet was used. The mean maximum residue concentrations observed at the end of the treatment at day 1 withdrawal with the 2.5 g/kg diet were 1.2 and 0.45 mg/kg for the medicated fish flesh and pellet diets, respectively. The corresponding figures for the residues for the 5 g/kg diet were 20.0 and 0.75 mg/kg. Residues in muscle tissue were detected up to 11 days and 3 days after withdrawal of the medicated fish-flesh and pellet diets, respectively. The half-life of oxytetracycline in prawns given the medicated fish flesh diet was 1.2 days (Limpoka et al., 1993). Summary of oxytetracycline tissue residue data is presented in Table 3.

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Table 2. **Mean prawn tissue and hemolymph concentrations of oxytetracycline (OTC) following a single forced oral administration of OTC at 11 and 22 mg/kg body weight**

Time (h)/ Dose	Hemolymph concentration (μg/ml)		Prawn tissue concentration (μg/g)			
	11 mg/kg	22 mg/kg	11 mg/kg		22 mg/kg	
0	0	0	0	0	0	0
0.5	[*]ND	[*]ND	[**]ND	[***]0.09±0.02	[**]ND	[***]0.10±0
1	ND	ND	ND	0.21±0.15	ND	0.26±0
2	ND	ND	ND	0.39±0	ND	0.52±0.06
4	ND	trace	ND	0.62±0	ND	0.82±0
8	0.5	1.0	ND	0.74±0.05	ND	0.97±0
12	trace	trace	ND	0.68±0	ND	0.90±0
24	ND	ND	ND	0.36±0	ND	0.55±0.04
30	ND	ND	ND	0.25±0	ND	0.41±0
48	ND	ND	ND	0.08±0.03	ND	0.18±0
54	ND	ND	ND	0.20±0.01	ND	0.20±0
72	ND	ND	ND	ND	ND	ND
96	ND	ND	ND	ND	ND	ND
120	ND	ND	ND	ND	ND	ND
144	ND	ND	ND	ND	ND	ND

[*] Assay by microbiological method, limit of detection for hemolymph = 0.2 μg/ml
[**] Assay by microbiological method, limit of detection for prawn tissue = 1.0 μg/g
[***] Assay by HPLC, limit of detection = 0.01 μg/g
ND = Not Detected

Exposure from the Environment

The Committee noted that prawns may be subjected to repeated exposure to oxytetracycline since sediments may be contaminated by medicated feed or by faeces containing the drug. In fish farming conditions, studies show that, following therapeutic use, oxytetracycline was found at concentrations of 0.1-10 mg/kg in the sediment. A significant portion of the drug was in a biologically inactive form, possibly as a result of the formation of complexes of oxytetracycline with divalent cations present in the aquatic environment. Therefore, exposure from sediments was not considered to be a major cocern.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

There are a variety of chemical assays available for the analysis of oxytetracycline in tissue and milk (FAO, 1990). In a paper by Oka et al. (1995), oxytetracycline, as well as other tetracyclines, were measured in beef liver using an high performance liquid chromatography (HPLC). The detection limit for oxytetracycline was 0.05 ppm. Levels as low as 1 ppb in bovine milk and meat have been attained using a tandem mass spectrometric approach (Traidi et al., 1985).

There are validated chemical assays available for the analysis of oxytetracycline in tissues of animals. The procedure which (Oka, 1985) employ EDTA and oxalic acid during extraction and clean up was extended for prawn and used in all of the previously-mentioned prawn studies. LOD is reported to be 0.01 mg/kg with 75% recovery.

Table 3. Summary of Oxytetracycline Muscle Tissue Residue Data in Giant Prawn (*Penaeus monodon*) Following Withdrawal of the Medicated Diets

Dose Forms	Maximum Dosage Regimen Tested	Method of Analysis	Average Oxytetracycline Residue Levels at Various Withdrawal Times (µg/g)										
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Powder OTC mixed in diet	2.5 g/kg in fish flesh diet for 5 days	Microbiological assay	1.20±0.50	<1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	1.20±0.13	1.0±0.09	0.5±0.02	0.35±0.03	0.23±0.01	0.19±0.04	0.10±0	0.07±0	0.05±0	0.03±0	ND
	5.0 g/kg in fish flesh diet for 5 days	HPLC	20.0±0	15.50±0	10.20±0.28	3.90±0	2.84±0	0.95±0.07	0.70±0.05	0.34±0.04	0.26±0	0.10±0	ND
Formulated medicated diet	2.5 g/kg in formulated pellets for 5 days	Microbiological assay	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	0.45±0.30	0.03±0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5.0 g/kg in formulated pellets for 5 days	Microbiological assay	<1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	0.75±0.03	0.35±0.02	0.10±0	ND	ND	ND	ND	ND	ND	ND	ND

Each value is mean of 6 individual prawn samples with standard deviation
Limit of detection of the microbiological assay is 1 µg/g
Limit of detection of the HPLC method is 0.01 µg/g
ND = Not Detected

Another modified HPLC method (Farrington et al., 1991) which is based on metal chelate affinity chromatography was reported to have a quantification limit of 0.01 mg/kg and 74% recovery.

The available microbiological assay has a limit of detection of 1 mg/kg in prawns tissues that are normally consumed. This assay is not, however, suitable for regulatory monitoring due to the low sensitivity.

APPRAISAL

An MRL of 100 µg/kg was recommended for muscle in all species at the thirty-sixth meeting of the Committee. At its forty-third meeting the Committee considered the human consumption of farmed prawns and adopted a daily intake value of 300 g of muscle and skin in natural proportions. The Committee recommended this value as an alternative to the meat consumption factor normally used.

At its present meeting, the Committee recommended a temporary MRL for oxytetracycline of 100 µg/kg for the edible tissue of giant prawn *(Penaeus monodon)*, expressed as parent drug. The MRL is temporary pending the availability of a validated analytical method. Such a method is required for review in 1996.

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TETRACYCLINE

First draft prepared by
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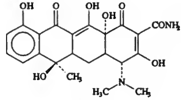
Australian Government Analytical Laboratory
Pymble, Australia

IDENTITY

Chemical names:	4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthalenecarboxamide
Synonyms:	Deschlorobiomycin; Tsiklomitsin; Abricycline; Achromycin; Agromicina; Ambramicina; Ambramycin; Bio-Tetra; Bristaciclina; Cefracycline suspension; Criseociclina; Cyclomycin; Democracin; Hostacyclin; Omegamycin; Panmycin; Polycycline; Purocyclina; Sanclomycine; Steclin; Tetrabon; Tetracyn; Tetradecin.

Structural formula:



Tetracycline

Molecular formula:	$C_{22}H_{24}N_2O_8$
Molecular weight:	444.43

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:	Tetracycline, tetracycline hydrochloride or the phosphoric acid complex of tetracycline
Major impurities:	Chlortetracycline, epitetracycline (8% max.)
Appearance:	Finely divided yellow powder
Melting point:	Trihydrate, crystals, swell at 165°, m.p. 170-175°(dec), becomes anhydrous by drying *in vacuo* at 60° for 8 hrs.
Optical rotation:	$[\alpha]^{25}_{D} = -239°$(in methanol)
Solubility:	Water 1.7 g/L, MeOH >20 g/L

Stability:	Tetracycline, tetracycline bydrochloride and the pbosphate complex of tetracycline are stable for more than 6 months at ambient temperatures.
Manufacture:	Chlortetracycline is antibacterial agent obtained by aerobic fermentation of strains of *Streptomyces aureofaciens* or *Streptomyces viridifaciens*. It is obtained commercially by large scale fermentation.
Mode of Action:	All tetracyclines act by the inhibiting attachment of aminoacyl-t RNA to the A site on the 30S ribosome to prevent protein synthesis. Translation is inhibited bv one molecule of the tetracycline per ribosome. It is postulated that a tetracycline-magnesium complex is formed at the ribosome, making it less flexible and therefore unable to bind aminoacyl-t RNA. It has been found that binding of tetracyclines to the 30S ribosome is dependent on proteins S7, S14 and S19 (Sande and Mandell, 1990).

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Tetracycline hydrochloride is a broad-spectrum antibiotic used in both buman medicine and veterinary medicine for treatment of infections caused by tetracycline sensitive organisms. It has been used in veterinary medicine for more than 40 years. Tetracycline has been used primarily as short-term, oral dosage formulations for the treatment of clinical diseases. This usage contrasts with that of chlortetracycline and oxytetracycline, antibiotics which bave been principally used as prophylactic agents in medicated feeds.

Chickens and Turkeys

Treatment of chronic respiratory disease; treatment of infection by tetracycline sensitive organisms; control of synovitis; control of coccidiosis; treatment of blue comb, infectious enteritis and sinusitis, mud fever and hexamitiasis.

Swine

Treatment of bacterial swine enteritis; treatment of infection by tetracycline sensitive organisms.

Cattle/Sheep/Horses

Treatment of infection by tetracycline sensitive organisms.

METABOLISM AND PHARMACOKINETICS

Metabolism

There is no definitive evidence of tetracycline metabolism *in vivo*. In common with otber members of the tetracycline group, tetracycline can be readily converted to microbiologically inactive isomers but these isomers are almost certainly artifacts produced by cbemical rather than biological processes.

Studies concerning the metabolism of tetracycline were conducted in rats and dogs using radiolabelled tetracycline (Kelly and Buyske, 1960). One rat received an intraperitoneal dose while a second rat was given a similar oral dose. Tables 1 and 2 show the cumulative excretion of radioactivity from rats following oral and intraperitoneal doses of labelled tetracycline.

Table 1. Cumulative Percent Radioactivity Excreted Following a Single Oral Dose of ^{14}C-labelled Tetracycline Hydrochloride (60 mg/kg) in the Rat

	%in Urine		%in Faeces		% Total
Hour	Rat A	Rat B	Rat A	Rat B	(Average)
0-24	2.7	3.8	24.3	10.7	20.7
24-28	3.7	4.2	71.5	78.6	79.0
48-72	3.8	4.5	71.9	78.8	79.5
72-96	3.9	4.5	72.2	79.0	79.8
96-120	3.9	4.5	72.2	79.3	79.9
120-144	3.9	4.5	72.2	79.4	80.0
144-168	3.9	4.5	72.2	79.5	80.1

Table 2. Cumulative Percent Excretion of Administered Radioactivity Following a Single Intraperitoneal Injection of 60 mg/kg Tetracycline.HCl in the Rat*

	% in Urine		% in Faeces		% Total
Hour	Rat C	Rat D	Rat C	Rat D	(Average)
0-24	28.0	59.3	8.5	23.8	59.8
24-28	37.6	61.6	52.2	25.3	88.4
48-72	40.1	62.1	52.9	25.9	90.5
72-96	41.1	62.5	53.3	26.1	91.5
96-120	42.1	62.7	53.5	26.4	92.4
120-144	42.6	62.9	53.6	26.5	92.8
144-168	43.0	63.2	53.7	26.8	93.3

*One rat received ^{14}C-labelled drug, the other tritium-labelled at the carbon number 7 position

The low urinary excretion of radioactivity following the oral administration as compared to that following the intraperitoneal dosing is to be noted. Also of interest is the surprisingly high percentage of the intraperitoneal dose eliminated in the faeces. Approximately 90% of the administered radioactivity in the rat was eliminated either by the urinary or faecal route. A significant portion of the remaining activity was bound as chelated tetracycline on the skeleton of the animal. A dilute formic acid extract of the radioactivity from the faeces, when submitted to countercurrent distribution in a butanol : formic acid : water system, resulted in a single peak which agreed with that obtained from pure tetracycline treated in a similar manner. Similar techniques applied to the unextracted urine from the rat gave identical results. These data show that with the exception of the metal chelate formation in the skeleton, tetracycline appears to be chemically unaltered by the rat.

Two beagle dogs each received a single oral dose of 25 mg of ^{3}H-labelled tetracycline hydrochloride/kg of body weight. Table 3 shows the cumulative urinary excretion of radioactivity.

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Table 3. Cumulative Percent Radioactivity and Microbiological Activity Excreted in the Urine Following a Single Oral Dose of 25 mg/kg ^{3}H-labelled Tetracycline Hydrochloride in the Dog

	Radioactivity		Microbiological Activity
	Dog A	Dog B	Dog A
0-24	2.0	2.7	2.1
24-28	2.8	3.1	2.7
48-72	--	3.2	2.8

Faecal measurements were not made in this study. Direct measurement of ^{3}H-labelled tetracycline and analysis after countercurrent separation of tetracycline from the urine in an orally dosed dog showed identical curves, indicating that no chemical transformation had occurred in the body of the dog. The cumulative excretion of microbiological activity is also given. It can be seen that the values obtained by microbiological and radiological methods are in close agreement, supporting the countercurrent distribution studies that little or no biotransformation of tetracycline occurred in the body of the dog.

Pharmacokinetics of Tetracycline

Studies involving the absorption of chlortetracycline in the presence of divalent cations such as calcium and magnesium have been extensive and have been reviewed earlier in this volume (see monograph on Chlortetracycline). It was found that the absorption of chlortetracycline was suppressed in the presence of divalent cations but that this effect could be lessened by co-administration of organic acids, such as citric acid, which were able to form complexes with these cations. Although tetracycline can also form strong complexes with divalent cations, the effect of cations on tetracycline absorption has received little mention in the literature. Some of the apparent inconsistencies in the literature reviewed below may be due to the effects of divalent cations, co-administered with tetracycline, on absorption efficiency.

Pharmacokinetics in Laboratory Animals

Tetracycline was administered to rats which had been fasted for 12 hours as single oral doses of 75 mg/kg (Berte and Vandoni, 1962). Tetracycline plasma concentrations were measured at 1, 2, 3, 4 and 6 hours after dosing. Peak levels, averaging 3.6 mg/L, occurred two hours after dosing and declined to 0.5 mg/L by 6 hours. Tetracycline tissue concentrations were highest in liver and kidney at all sampling times. Residue levels in the lung tissue and plasma were of the same order whereas very low levels were found in brain tissue. Results are summarised in Table 4.

Table 4. Tetracycline Concentrations in Tissues and Plasma of Rats Following a Single Oral Tetracycline Dose of 75 mg/kg of body weight

Tetracycline Concentration, mg/kg					
Time After Dosing (h)	Plasma	Lungs	Brain	Liver	Kidney
1	3.1±0.7	3.7±1.4	0.12±0.02	8.5±1.1	11.0±1.6
2	3.6±1.4	4.0±0.8	0.13±0.05	10.1±2.6	12.8±2.6
3	2.1±0.77	1.7±0.9	0.02±0.01	4.0±0.7	8.7±0.5
4	2.2±0.75	1.5±0.9	0.01±0.01	3.0±0.08	4.5±0.5
6	0.5±0.45	1.2±1.1	0.01±0.0001	2.5±2.4	2.6±1.9

Beagle dogs were given single oral tetracycline doses of 25 mg/kg live weight. Peak serum levels of tetracycline, averaging 2.98 mg/L, occurred 2 hours after dosing, and declined to an average of 0.27 mg/L by 24 hours post dosing (Kanegis, 1958). Excretion in the urine accounted for 9.8% of the administered dose by 72 hours after dosing. In this same study, dogs given single IV tetracycline doses of 10 mg /kg attained average serum levels of 10.6 mg/L one hour postdosing, declining to 1.3 mg/L at 24 hours and 0.14 mg/L at 48 hr. Tetracycline levels, determined by microbiological assay, are summarised in Table 5.

Table 5. Average Blood and Urine Concentrations of Tetracycline in Dogs Given Single Oral Doses (25 mg/kg) or Single Intravenous Doses (10 mg/kg)

	Oral Dosing			Intravenous Dosing	
Hours After Dosing	Blood (mg/L)	Urine (mg/L)	Cumulative Recovery in Urine	Blood (mg/L)	Cumulative Recovery in Urine
1	2.05	63.0	0.35%	10.6	7.9%
2	2.98	301.0	1.4%	8.7	13.7%
4	2.38	340.0	2.9%	7.8	20.2%
6	1.88	201.0	4.1%	6.5	27.9%
8	1.41	320.0	5.1%	5.6	32.4%
24	0.27	36.0	8.2%	1.3	51.9%
48		7.7	9.7%	0.14	57.0%
72		1.1	9.8%		57.8%

^{3}H-labelled tetracycline hydrochloride was administered IV to two beagle dogs at 10 mg/kg to determine its distribution throughout the body (Kelly, 1964). Concentrations of tetracycline in the various tissues and fluids were determined by radioassay. Where it was possible to obtain the entire organ or tissue, the total antibiotic content within the tissue was calculated for the major tissues and is shown in summary form in Table 6. Four hours after dosing the organs containing the most tetracycline were the liver and kidney. A large proportion of the recovered antimicrobial activity was found in urine, intestinal contents and bile. With the exception of fat and spinal cord, radioactivity was found throughout all tissues and fluids examined.

Table 6. Distribution of Tetracycline in Tissues and Fluids of Dogs Four Hours After an Intravenous Dose of 10 mg TC/kg Live Weight

	Tetracycline, mg/kg		Total Tetracycline Content, μg	
Tissue or Fluid	Dog 98	Dog 78	Dog 98	Dog 78
Blood	NM	6.4	NC	NC
Liver	13.5	15.9	4,666	2,752
Kidney	14.1	75.8	776	3,024
Heart	3.3	5.7	315	297
Lungs	3.0	5.1	259	221
Brain	0.21	0.42	17	29
Skeletal Muscle	1.9	3.4	NC	NC
Femur-epiphysis	9.5	16.9	NC	NC
Femur-diaphysis	4.9	7.4	NC	NC
Bile	112.5	157.8	1,350	1,200
Spleen	3.5	5.7	56	84
Pancreas	6.0	3.0	119	35
Adrenals	0.9	2.6	NC	NC
Subcutaneous Fat	*	ND	NC	NC
Perirenal Fat	ND	*	NC	NC
Uterus	1.7	2.5	14	13
Trachea	NM	1.7	ND	12
Esophagus	1.6	2.2	41	38
Stomach	1.4	2.5	164	190
Duodenum	2.0	2.9	177	195
Jejunum	1.8	3.8	254	143
Ileum	1.9	3.5	197	186
Cecum	1.4	3.1	29	16
Colon	2.6	3.4	92	95
Rectum	1.6	2.4	54	29
Stomach Contents	1.5	0.9	14	35
Duodenal Contents	26.1	30.3	248	124
Jejunal Contents	37.9	28.2	459	592
Ileal Contents	187.0	202.0	1,552	384
Colon Contents	335.1	316.8	1,173	1,188
Rectal Contents	10.6	5.0	636	113
Thymus	2.3	2.0	NC	NC
Urinary Bladder	1.5	2.5	NC	NC
Diaphragm	3.1	3.0	88	160
Salivary Gland	1.7	3.4	NC	NC
Teeth	2.1	3.6	NC	NC
Urine	102.6	82.5	18,468	4,520
Spinal Cord	*	*		

NM = Not Measured; NC = Not Calculated; * = No detectable radioactivity present

Studies in rats, mice and rabbits have shown that tetracycline has a great affinity for bones and teeth. Under UV light, a brilliant yellow-gold induced fluorescence was observed almost instantaneously after IV administration in diffusely distributed tissues (with the exception of the brain) and within 30 minutes after IP injection (Milch et al, 1957). Fluorescence disappeared from all tissues except bone within 6 hours after a single parenteral injection. Bone fluorescence persisted throughout the 10-week period of observation following a single parenteral dose of tetracycline. Indeed, this property continues to be frequently used as a qualitative guide to previous exposure of an animal to tetracyclines.

Deposition of tetracycline in bone has also been studied quantitatively (Buyske et al, 1960). Unlabelled and ^{3}H-labelled tetracycline was administered to rats and the results of part of this work in which various ^{3}H-labelled tetracycline doses were administered to rats by single IP injections are shown in Table 7. A direct relationship was found between tetracycline dose and residue concentrations in blood and bone.

A single oral dose of 250 mg ^{3}H-labelled tetracycline was administered to male rats in a further experiment from the same publication. The results of this study showed a much lower concentrations of tetracycline in the bone (ie, 1.9 mg/kg at 24 h, 0.4 mg/kg at 4 weeks) than found in the previous study with intraperitoneal dosing.

Table 7. Concentration of Tetracycline in the Serum and Femora of Male Rats Following a Single Intraperitoneal Dose

	Tetracycline Concentrations, mg/L or mg/kg				
	Serum				Bone
Dose, mg/kg	2 h	4 h	6 h	24 h	24 h
150	48	54	47	35	186
100	38	38	31	15	140
65	28	17	21	4	112
45	21	18	15	1.4	91
30	9	7	4	0.2	17
20	8	6	3	0.1	12
10	6	3	1.9	<0.1	10

Single IV doses of ^{3}H-labelled tetracycline (15 mg/kg) were given to two rats with cannulated bile ducts and indwelling catheters in the ureters to measure the relative importance of bile in the excretion of tetracycline from the body. Radioactivity was measured in urine, bile and gastrointestinal tract contents for 24 hours after dosing. A summary of the results are presented in Table 8.

In another study, two rats and one beagle dog were given single intravenous doses of ^{3}H-labelled tetracycline hydrochloride (Eisner and Wulf, 1962). The results of this work, presented in Table 9, shows the percentage of the dose excreted via the urine and faeces.

Table 8. Excretion of Radioactivity by the Rat and the Dog Following Intravenous Administration of ^{3}H-labelled Tetracycline Hydrochloride

Species	Number of Animals	Dose mg/kg	Hours After Dose	Urine	Faeces	Total
Rat	2	15	0-72	69.2	19.5[a]	88.7
Dog	1	4	0-168	71.0	9.0	80.0

a = 0-48 hours

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Table 9. Excretion of Radioactivity from ^{3}H-labelled Tetracycline by Rats with Chronic Bile Duct Canulae for 24 Hours Following an IV Dose of 15 mg/kg

	Volume, mL			% of Dose		
Rat Number	Urine	Bile	Recovery of Dose, (%)^a	Urine	Bile	GI Tract
1	9.3	18.5	69.5	67.8	29.8	2.4
2	20.5	14.5	85.0	88.3	8.9	2.7

a = % of radioactivity from dose recovered in urine, bile and gastrointestinal tract contents over 0-24 hours after dosing

Although the results from the two rats did not agree well with each other, this experiment did indicate that relatively small amounts of tetracycline were excreted into the intestine by means other than the biliary route. A simpler experiment was also conducted in which the bile ducts of three rats were ligated. Whole blood, plasma and gastrointestinal tract contents were measured for radioactivity and microbiological activity following a single IV dose of 15 mg/kg of ^{3}H-labelled tetracycline. The results are summarised in Table 10.

Table 10. Blood Concentration and Urinary Excretion of Tetracycline by Rats with Ligated Bile Ducts Following an Intravenous Dose of 15 mg/kg ^{3}H-labelled Tetracycline

	Whole Blood, mg/L		Plasma, mg/L	
Hours	Microbiological	Radioactivity	Microbiological	Radioactivity
4	3.96	6.44	6.62	7.36
8	3.05	3.80	4.54	5.28
24	1.66	2.65	2.54	3.03
	% Recovery in Urine		% Recovery in GI Tract	
Hours	Microbiological	Radioactivity	Radioactivity	
0-4	35.3	36.3	1.7	
0-8	38.8	40.3	1.9	
0-24	47.6	49.1	4.8	

Less than 5% of the administered radioactivity was recovered from the gastrointestinal tract contents over the 24-hour period following dosing, while 49.1% of the administered dose was recovered in the urine. Tissues of these animals were not examined, so no conclusions can be made regarding total recovery of the administered dose. There is good agreement between the microbiological and radioactivity results for urine and blood, supporting the conclusions of the metabolism studies (Kelly and Buyske, 1960) which showed that little or no biotransformation of the tetracycline molecule had taken place in the body of rats and dogs which were used in the studies.

The absorption of tetracycline hydrochloride excreted in the bile of rats was evaluated using an *in situ* intestinal preparation (Adir, 1975). For comparative purposes, the absorption of the drug from an aqueous solution having the same pH as that of the bile was also determined. After 4 hours, the amounts of tetracycline absorbed from the bile and aqueous solutions were 72.9 and 77.3% respectively. There was no significant difference in the amount of drug accumulated in the gut tissue. The disappearance of the drug from the intestinal lumen was biexponential and the kinetic parameters appeared to be similar. It was concluded that tetracycline excreted in the bile is readily absorbed from the rat intestine. Accordingly, biliary excretion does not seem to account for a significant elimination of this antibiotic from the body. The findings of this investigation raise questions regarding the other routes of elimination of tetracycline in addition to excretion in urine.

Pharmacokinetics in Pigs

Five Yorkshire x Hampshire crossbred pigs averaging 95 pounds body weight were given drinking water containing 600 mg of tetracycline hydrochloride/gallon for five consecutive days (Schumacher, 1968). Average daily intake of tetracycline during the 5-day medication period was 13.8 mg/kg. Average tetracycline levels in the blood and urine, measured periodically during and after medication, are shown in Table 11.

Table 11. **Average Concentrations of Tetracycline in Blood and Urine of Pigs During and After Consumption of Drinking Water Containing 600 mg Tetracycline per Gallon for Five Consecutive Days**

	Treatment Phase on Medication			**Off Medication**	
	Tetracycline, mg/L			Tetracycline, mg/L	
Time (h)	**Blood**	**Urine**	**Time (h)**	**Blood**	**Urine**
6	0.29	NS[1]	6	0.16	NS
12	0.33	NS	12	0.08	NS
24	0.33	9.6	24	ND[2]-0.06 (2/5)[3]	2.87
48	0.14	16.8	48	ND-0.05 (1/5)	2.21
72	0.16	19.8			
96	0.22	9.7			
120	0.37	13.4			

[1] NS = Not Sampled; [2] ND = Not Detected, less than 0.06 mg/L; [3] (2/5) = Number of pigs with detectable value of tetracycline

It is apparent from these data that tetracycline is rapidly absorbed by pigs when administered via drinking water, reaching a peak of 0.33 mg/L of blood by 12 hours on medication. The concentrations of tetracycline are much higher in the urine than in the blood, and tetracycline continues to be found in the urine after it is no longer detectable in the blood.

Two studies were conducted to assess the bioavailability and pharmacokinetics of tetracycline fed to swine (Kniffen et al, 1989). In the first of these, four Yorkshire x Chester White gilts weighing approximately 32 kg were used in a 2 x 2 crossover design. In the first phase, two gilts were dosed with 22 mg/kg of an aqueous solution of tetracycline hydrochloride by stomach tube after being fasted for 16 to 20 hours, while the remaining two gilts were administered 11 mg/kg via an indwelling arterial cannula. Blood samples were obtained at predetermined intervals over the 72 hours following dosing. One week after completion of each of the initial phases of the trial, the gilts which had been dosed orally were dosed intra-arterially and the gilts which had been dosed intra-arterially were dosed orally. The results of the area under the curve measurements, shown in Table 12, indicate that tetracycline was poorly and variably absorbed after oral administration. The mean F value was 0.23, which corresponded to a bioavailabiltiy of 23%.

Table 12. **Area Under the Plasma Tetracycline Concentration-time Curve (AUC) Values after Intravascular (IV) and Oral Administration of Tetracycline Hydrochloride to Swine at the Dosage of 11 and 22 mg/kg, respectively**

	Gilt Number					
Variable (units)	1	2	3	4	Mean	SD
AUC IV (h·mg/L)	50.20	59.40	65.00	66.00	60.15	7.24
AUC Oral (h·mg/L)	10.60	32.40	37.40	32.60	28.25	11.99
F*	0.11	0.27	0.29	0.25	0.23	0.08

*F (fraction absorbed) was calculated as the dose-corrected ratio of the oral to-intravascular area under the curve

Pharmacokinetic variables describing the disposition of tetracycline in gilts indicated that after intravascular administration of tetracycline hydrochloride, plasma concentration of tetracycline decreased in a triexponential fashion (Table 13).

The investigators concluded that for all gilts, a triexponential equation provided a better fit to the data than did a biexponential equation. The pharmacokinetic behaviour of the drug can best be described by a 3-compartment open model. The terminal elimination phase (g) rate constant of 0.043 ± 0.01 /h corresponded to a biological $t_{1/2}$ of approximately 16 hours. The large Vd_{area} of 4.5 + 1.06 L/kg suggested that tetracycline was widely distributed in swine tissues. After oral administration of tetracycline hydrochloride, the resulting plasma concentrations of tetracycline were too variable to be fitted to a rational absorption curve.

These authors also fed a ration containing 550 mg/kg of tetracycline hydrochloride to three gilts. Tetracycline plasma concentrations were determined at selected times during 96 hours after exposure to the medicated feed and were found to reach a fairly constant value of 0.3-0.5 mg/L after 4 hours after start of medicated feeding.

Table 13. **Pharmacokinetic Variable for the disposition of Tetracycline in Gilts after Intra-arterial Administration of Tetracycline Hydrochloride (11 mg/kg)**

	Gilt Number					
Variable (units)	1	2	3	4	Mean	SD
A (mg/L)	78.10	69.80	95.50	90.20	83.40	11.63
B (mg/L)	7.52	6.89	7.80	8.78	7.75	0.79
C (mg/L)	0.861	0.750	1.60	0.623	0.959	0.439
a (/h)	18.50	10.00	11.70	13.70	13.48	3.68
b (/h)	0.307	0.221	0.227	0.243	0.250	0.039
g (/h)	0.0436	0.0382	0.0579	0.0321	0.0430	0.0110
Cl (L/kg/h)	0.219	0.185	0.169	0.167	0.185	0.024
Vd_{area} (L/kg)	5.03	4.85	2.92	5.19	4.50	1.06
K (/h)	1.78	1.34	1.50	1.60	1.56	0.18
Vp (L)	0.127	0.142	0.105	0.110	0.121	0.017

A, B, and C = zero time plasma drug concentrations; a, b, and g = disposition rate constants; Cl = clearance; Vd_{area} = volume of distribution; K = elimination rate constant; Vp = volume of central compartment.

Six Yorkshire pigs, weighing 46 to 83 kg and fitted with jugular vein catheters, were given drinking water containing 400 mg/L tetracycline for 3 days (Luthman et al, 1989). After a resting period of one week, the study was repeated with drinking water containing 800 mg/L tetracycline. Serum levels, measured at 2-hour intervals during the day, were highest in each animal in the late evening, followed hy a decrease during the night. The highest serum levels with 400 mg/L tetracycline were 0.30 to 0.47 mg/L, while the corresponding tetracycline levels with the 800 mg/L treatment were 0.65 to 1.15 mg/L. Six other pigs with a mean weight of 25 kg were given feed containing 1000 mg/kg tetracycline for nine days. The pigs were fed twice daily at 07.00 and 16.00. Blood samples, taken at 2-hour intervals between 09.00 and 19.00, showed mean serum levels ranging from 0.13 to 0.20 mg/L. In a third experiment, seven pigs were given single oral doses of 40 mg/kg tetracycline. Five of the pigs were fasted overnight prior to dosing while two pigs were fed 2 hours prior to tetracycline administration. Feeding was not allowed during the 24-hour sampling period after dosing. Tetracycline serum levels were highest 2 to 4 hours after dosing and peak levels were less than 1 mg/L for the fed pigs compared to about 1.5 to 5.0 mg/L for the fasted pigs. It is clear that feeding significantly reduced the bioavailability of orally administered tetracycline in these pigs.

Groups of six pigs weighing approximately 15 kg received drinking water containing 147 mg/kg of tetracycline continuously for 1, 3 and 7 days (Rooney, 1990). Resulting average blood and lung concentrations are shown in Table 14.

Table 14. Average Tetracycline Levels in blood and Lung Tissues from Pigs Receiving Drinking Water Containing 147 ppm of Tetracycline

	Tetracycline, mg/L	
Time (days)	Blood	Lung
1	0.262	0.372
3	0.268	0.373
7	0.256	0.366

Concentrations of tetracycline in the lung tissues averaged 39 to 43% higher than those found in the blood.

Pharmacokinetics in Cattle and Sheep

Calves approximately one month of age were used to study the bioavailability of tetracycline (Luthman et al, 1989). The calves were fed 2 litres of a conventional milk replacer twice daily and had free access to hay. In addition, about 0.3 kg of a milk replacer was offered shortly after the first daily milk feeding, most of which was consumed in 3-4 hours. The calves were fed 25 mg/kg bw mixed into the milk replacer twice daily. The calves were treated in this manner for five days. Blood was sampled on days 1, 2 and 5 as shown in Table 15.

Table 15. Mean Serum Concentrations (mg/L) of Tetracycline in Calves Fed 25 mg/kg b.w. Twice Daily of Tetracycline in the Milk Replacer

	Time of Day				
	10.00	12.00	16.00	18.00	20.00
Day 1	1.78±0.26	1.85±0.27	1.35±0.39	2.25±0.30	
Day 2	0.95±0.18				
Day 5	2.27±0.32	2.10±0.32		2.23±0.30	2.10±0.34

The same authors also conducted a second study in which calves were given 50 mg /kg bw of tetracycline in the milk replacer at the first morning feeding. After one week the same dose was given in 1 litre of water 4 hours after the morning feeding. The calves were fed concentrate and had access to hay during the whole sampling period. Blood was sampled after 1, 2, 4, 6, 8 and 10 hours. Serum tetracycline levels were significantly higher for the calves receiving tetracycline in the milk replacer than after the same dose given in water 4 hours after the first daily milk feed. C_{max} was achieved after 4 hours in all animals. It was postulated that the 0.3 kg of concentrate consumed prior to the dose of tetracycline in water may have been responsible for the lowered bioavailability of the tetracycline.

Tetracycline was administered by a rapid IV injection into the jugular vein of six Israeli-Friesian cows and four Awassi ewes at a dose of 20 mg/kg of bw, and periodic blood samples were obtained over the 72 hours following dosing (Ziv and Sulman, 1974). As concentrations of tetracycline in the blood serum (determined by microbiological assay) were similar for cows and ewes, the data were presented as the combined data. Venous blood concentration curves of all cows and sheep in this experiment were biexponential, and the data appeared to be adequately described by the 2-compartment open model. Pharmacokinetic values calculated from the data are presented in Table 16.

Table 16. Pharmacokinetic Constants for Tetracycline Administered Intravenously to Cows and Ewes at 20 mg/kg of Body Weight

Value	Definition and Method of Calculation	Mean	SD
A μg/ml	Extrapolated zero-time serum drug concentration of the α-phase	20.5	3.6
B μg/ml	Extrapolated zero-time serum drug concentration of the β phase	12.5	1.7
C_p° μg/ml	A + B	33.0	4.8
α b^{-1}	Slope of the initial phase of serum TC concentration	1.236	0.14
β h^{-1}	Slope of the second phase of serum TC concentration	0.054	0.01
k_{12} b^{-1}	First-order distribution rate constant between the central compartment and the peripheral compartment = $AB(\beta-\alpha)^2/\ A\beta+B\alpha\cdot(A+B)$	0.655	0.21
k_{21} h^{-1}	First-order distribution rate constant between the peripheral compartment and the central compartment = $(A\beta)+(B\alpha)/A+B$	0.502	0.08
k_{el} h^{-1}	Overall rate constant for TC elimination by various routes = $(\alpha\beta)\cdot(A+B)/A\beta+B\alpha$	0.121	0.04
$t\frac{1}{2}k_{el}$ h	Half-life of overall rate constant for drug elimination = $0.693/k_{el}$	5.73	0.6
V_p % bw	Specific total volume of distribution = V_p/kg of body weight 100	332.2	23.5
F_c	Fraction of the drug in the body located in the central compartment = $\beta\ /k_{el}$	0.45	0.06

In another study, a solution containing a mixture of 100 mg of unlabelled tetracycline and 0.8 mCi of ^{3}H-labelled tetracycline (3 mCi/mg) was injected into the right front quarter of an Israeli-Friesian dairy cow after a normal morning milking (Ziv et al, 1974). Milk samples and blood samples were taken at hourly intervals for 8 hours after treatment. The concentration of tetracycline in the treated quarter decreased exponentially during the first 7 to 8 hours after treatment. The rates of decrease of radioactivity and of tetracycline by microbiological assay were essentially parallel. Antibiotic activity was not detectable in the blood serum or in the milk of the untreated quarters after treatment. However, radioactivity counts, 3 to 6 times greater than the background counts, were recorded in the serum and in milk from the nontreated quarters from 1 to 8 hours after the radioactive-labelled tetracycline was injected into the treated quarter. It appears that the principal mode of transfer of tetracycline from treated to untreated quarters was via the bloodstream.

Pharmacokinetics in Poultry

Two experiments by Anadon, et al. (1985) studied the pharmacokinetics of tetracycline in chickens. Forty to sixty-day-old broiler chickens weighing 1.5 to 2.0 kg were used. In the first experiment, tetracycline hydrochloride was injected into the left brachial vein of each of a group of six chickens at a single dose of 65 mg/kg bw. Plasma tetracycline concentrations, measured by HPLC analysis, are shown in Table 17. The pharmacokinetic parameters which describe the distribution and elimination phases of tetracycline after the 65 mg/kg dosage are given in Table 18.

Table 17. Plasma Concentrations (mg/L) of Tetracycline in Chickens Receiving an Intravenous Dose of 65 mg/kg of Body Weight

Time after Administration	Tetracycline, mg/L	
	Mean	SEM
15 min	690	17
30 min	359	12
1 hour	81	5
2 hours	52	6
4 hours	29	4
8 hours	12	2.3
12 hours	3.8	0.9

Table 18. Pharmacokinetic Parameters for Tetracycline Administrated IV at 65 mg/kg of Body weight to Chickens

Parameters	Mean
A, mg/L	2000±450[1]
B, mg/L	82±6
α, h^{-1}	4.274±0.489
ß, h^{-1}	0.252±0.009
$t_{½}\alpha$, h	0.162
$t_{½}$ß, h	2.772
VC=Central compartment, L/kg	0.037
Peripheral compartment, L/kg	0.137
Total vol of distribution, L/kg	0.174
K_{12}, h^{-1}	1.497
K_{21}, h^{-1}	0.408
K_{10}, h^{-1}	2.614
Conc. time curve, mg/h/kg	796.38
Total body clearance, L/h/kg	0.0979
K_{12}/K_{21}	3.667
K_{12}/K_{10}	0.573
K_{21}/K_{10}	0.156

[1] Mean ± standard error for 6 chickens

In the second experiment, bile ducts were cannulated with polyethylene tubing. Two groups of 3 chickens each were given a single IV injection of tetracycline at doses of 10 mg/kg and 15 mg/kg, respectively. Two other groups of chickens received single oral doses of 100 mg/kg and 200 mg/kg of tetracycline. Tetracycline was excreted into the bile following both IV and oral administration. The biliary excretion data after IV administration are shown in Table 19.

Table 19. Biliary Excretion Rates ($\mu g/h$) of Tetracycline (mean ± SEM) After Intravenous Administration in Chickens

	Tetracycline Dose	
Time after Administration (h)	10 mg/kg	15 mg/kg
0.5	225.0±21.0	329.0±17.3
1	407.0±10.8	606.0±36.2
2	308.6±36.2	275.3±13.8
3	44.8±5.6	118.0±13.1
4	24.0±3.8	70.4±8.5
5	15.7±1.9	39.1±2.9
6	8.0±1.7	20.5±4.9

With both of the doses there was a log-linear decline of bile concentration indicative of a one compartment model. The investigators calculated that with the IV doses used, the median rate constants for drug biliary excretion were 0.834 and 0.665 h^{-1}, for the 10 and 15 mg/kg doses, respectively. The corresponding median half-lives were calculated to be 0.831 and 1.05 hours. During the 6 hour period following IV administration, 7% of the administered tetracycline was excreted into the bile. After oral administration, the percentages of the total doses recovered from the bile were much lower than following IV administration. The average maximum cumulative amount of tetracycline excreted into the bile with 8 hours was 260 and 1480 μg after oral administration of 100 and 200 mg/kg respectively, which corresponds to 0.2 and 0.5% of the total doses administered.

The pharmacokinetics of tetracycline was studied in turkeys (Gonzelman a et al., 1985). In the first of these experiments, five Broad-breasted White turkeys weighing 3 to 4 kg were used. Feed was withheld for 12 hours before dosing, but the birds had free access to water. After collection of pretreatment blood samples, 25 mg/kg bw of tetracycline hydrochloride, buffered with ascorbic acid, was injected intravenously. Blood samples, taken periodically after dosing, were assayed microbiologically for tetracycline content. Results of this work are shown in Tables 20 and 21.

Table 20. Mean (± SD) Concentration (mg/L) of Tetracycline in Serum Samples Collected After a Single Intravenous Injection of Tetracycline Hydrochloride at 25 mg/kg Body weight

Time After Injection (min)						
5	15	30	60	90	127	232
47.4±9.0	27.4±7.2	16.7±1.8	12.9±2.2	9.5±2.4	6.7±2.6	5.0±2.5

Kinetic parameters calculated from these data are shown in Table 21.

Table 21. Pharmocokinetics of Tetracycline in Turkeys Following Intravenous Administration of a Single 25 mg/Kg Dose of Tetracycline Hydrochloride

Kinetic Parameter	Units	Measurement	Kinetic Parameter	Units	Measurement
A	mg/L	62.8±25	k_{el}	min^{-1}	0.0230±0.0115
B	mg/L	15.8±4.3	V_c	ml/kg	345±136
α	min^{-1}	0.1210±0.057	$t_{0.5}$	h	2.41±1.03
β	min^{-1}	0.0054±0.002	$Vd_{(area)}$	ml/kg	1377±373
k_{12}	min^{-1}	0.0742±0.04	Cl_B	ml/min-kg	7.70±1.9
k_{21}	min^{-1}	0.0293±0.0125	$AUC_{(IV)}$	µg·h/ml	59.74

In a second experiment, nine turkeys of similar weight to those used in the first experiment were given a single oral dose of tetracycline hydrochloride at 25 mg/kg. The drug solution (5 mg/ml) was delivered into the proventriculus with a feeding tube. Table 22 shows tetracycline content of blood samples taken by venipuncture over 12 hours after dosing.

Table 22. Mean (±SD) Concentrations (mg/L) of Tetracycline in Serum Samples Taken After A Single Oral Dose of Tetracycline HCl at 25 mg/kg

Time After Administration (hours)											
0.25	0.50	0.75	1.0	1.5	2	3	4	6	8	10	12
2.4	3.3	3.4	3.8	3.3	3.0	2.4	1.7	1.0	0.55	0.34	0.17
±.45	±.71	±1.0	±.96	±1.0	±.96	±.80	±.54	±.68	±.32	±.12	±.12

The pharmocokinetics parameters are shown in Table 23.

Table 23. Pharmacokinetics of Tetracycline in Turkeys Following a Single Oral Dose of Tetracycline Hydrochloride at 25 mg/kg of Body Weight

Kinetic Parameter	Units	Measurement
C_0	µg/ml	5.07±1.4
(hypothetical) k_a	h^{-1}	3.0963±1.189
$t_{0.5(a)}$	h	0.265±0.14
k_{el}	h^{-1}	0.3086±0.087
$t_{0.5(apparent)}$	h	2.44±0.77
AUC_{po}	µg·h/ml	5.29
F[1]	%	9

[1] F = $(AUC)_{po}/(AUC)_{iv}$

The authors stated that the pharmacokinetic parameters determined above suggest that effective serum levels of tetracycline may be difficult to attain by admixture of the drug in drinking water. Although the rate of absorption is rapid, the extent of the absorption is incomplete (9% of the administered dose), and the elimination rate is rapid. The authors calculated that a concentration of 8.5 g/L of drinking water would be expected to provide serum concentrations of 0.5-1.0 mg/L.

Summary of Pharmacokinetics Across Species

The studies reported above show that tetracycline is rapidly absorbed following oral administration in all species, but the degree of absorption is poor. Fasting of the animal overnight prior to administering an oral dose of tetracycline has been shown to increase absorption. Numerous studies with chlortetracycline and oxytetracycline have shown that the presence of calcium in a medicated feed will cause a decrease in the bioavailability of these tetracyclines, and it would be expected that calcium in the dosing medium or in the stomach of the animal would have the same effect on the bioavailability of tetracycline. Tetracycline is widely distributed throughout the body soon after dosing, and with the exception of those residues which are bound to bone, is rapidly excreted. The half-life of a dose of tetracycline in poultry appears to be shorter than in calves and swine. The primary route of excretion of tetracycline from the body is via the urine, but considerable amounts are also excreted via the faeces. It is believed that excretion via the bile accounts for the main route for absorbed tetracycline to get into the faeces. However, studies have shown that tetracycline excreted in the bile is readily available for reabsorption back into the body.

TISSUE RESIDUE DEPLETION STUDIES

General

Data from pharmacokinetic studies in laboratory animals and in target species, and metabolism studies in laboratory animals have shown that tetracycline is rapidly, but poorly, absorbed following oral administration. It is widely distributed throughout the body and, with the exception of those residues which are bound to bone, is rapidly excreted. Metabolism studies in rats and dogs indicate that little or no biotransformation of the tetracycline molecule occurs within the body. Unchanged tetracycline is eliminated primarily via the urine, but considerable amounts are also excreted in the faeces. Most of the residue data have been obtained using microbiological assay procedures. Recently, the development of HPLC methods has made available an alternative method for residue monitoring. The available residue data for the use of tetracycline hydrochloride in target species are summarised in the following section.

Depletion of Residues from Edible Tissues of Swine

Yorkshire x Hampshire crossbred pigs averaging about 20 kg in weight were offered drinking water containing 750 mg/gallon of tetracycline hydrochloride for 14 consecutive days (Berger, 1972). The calculated average daily intake of tetracycline was 24.2 mg/kg over the 14-day medication period. Tissue samples taken periodically after withdrawal of medicated drinking water were assayed microbiologically for residues of tetracycline. Results are summarised in Table 24.

Table 24. **Average Residue Levels of Tetracycline in Blood and Tissues of Swine at Various Withdrawal Times After Receiving Drinking Water Containing 750 mg/gallon of Tetracycline HCl for 14 Consecutive Days**

	Tetracycline Residues (mg/L or kg)				
Withdrawal Day	**Blood**	**Muscle**	**Liver**	**Kidney**	**Fat**
0	0.89	0.71	1.76	3.43	ND-0.078
4	ND-0.055	0.096	ND-0.131	0.241	ND
7	ND[1]	ND-0.085	ND-0.097	0.147	ND
10	ND-0.05	ND	0.10	0.170	ND
14	ND	ND	ND-0.076	ND-0.089	ND

[1] ND = Not Detected, less than sensitivity of microbiological assay method (0.06 mg /L)

Depletion of Residues from Edible Tissues of Cattle

Jersey heifer calves, average weight 76 kg, were treated by oral drench with 400 mg of tetracycline hydrochloride per head daily for 5 consecutive days (Berger, 1967). The calves were sacrificed at 0, 2, 5 and 7 days following withdrawal of treatment for assay for tetracycline content of tissues and fluids by microbiological assay. The results of these assays are summarised in Table 25.

Table 25. **Average Residues of Tetracycline in Tissues and Fluids from Calves at Various Withdrawal Times after Receiving 400 mg Tetracycline/Calf/Day for Five Consecutive Days**

	Tetracycline Residue (mg/kg)					
Withdrawal Day	**Muscle**	**Liver**	**Kidney**	**Fat**	**Blood**	**Urine**
0	ND-0.17	0.38	0.71	ND	0.10	10.47
2	ND[1]	ND	ND-0.12	ND	ND	0.91
5	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND

[1] ND = Not Detected, less than sensitivity of microbiological assay method (0.06 mg/L for blood and 0.1 mg/kg for tissues); Bile was found to give false positive results in the microbiological assay

In another study bull calves with an average weight of 68 kg were treated with tetracycline hydrochloride by oral drench daily for 10 consecutive days at the dose of 22 mg/kg b.w. per day (Berger, 1975). Tissue and blood samples were assayed for tetracycline residue content by microbiological assay at various withdrawal times following cessation of treatment. The results of these assays are summarised in Tables 26 and 27.

Table 26. **Average Residues of Tetracycline in Tissues of Calves at Various Withdrawal Times After Cessation of Treatment with Tetracycline at 22 mg/kg bw for 10 Consecutive Days**

	Tetracycline, mg/kg_			
Withdrawal Day	**Muscle**	**Liver**	**Kidney**	**Fat**
2	ND[1]	0.21	0.33	ND
4	ND	ND-0.12	0.17	ND
8	ND	ND	ND-0.12	ND
12	ND	ND	ND-0.13	ND
15	ND	ND	ND	ND

[1] ND = Not Detected, less than sensitivity of microbiological assay method (0.10 mg/kg)

Table 27. Average Residues of Tetracycline in Blood of Calves at Various Times Following Cessation of Treatment at 22 mg/kg bw for 10 Consecutive Days

Time Following End of Treatment	Tetracycline Average, mg/L	Time Following End of Treatment	Tetracycline Average, mg/L
Hours		Days	
2	1.03	2	0.09
4	0.79	3	0.06
8	0.62	5	ND-0.04
12	0.45	7	ND-0.03
24	0.20	9	ND-0.03
		11	ND[1]
		13	ND
		15	ND

[1] ND = Not Detected, less than sensitivity of microbiological assay method (0.0375 mg/L)

In another experiment, Holstein bull calves approximately 2 weeks of age, average weight 41.6 kg, were treated for ten consecutive days with tetracycline HCl oral boluses to furnish 22 mg/kg bw daily (Eggert, 1978a). The calves were fed a nonmedicated milk replacer twice daily during the experiment. The boluses were administered each day following the morning feeding. Three calves each were sacrificed at 0, 3, 7 and 10 days following completion of the treatment for analysis of residues of tetracycline in blood and tissue samples. The average results are summarised in Table 28.

Table 28. Average Residues of Tetracycline in Blood and Tissue Samples from Calves Following Cessation of Treatment with Tetracycline·HCl-Boluses at 22 mg/kg bw for 10 Days

	Tetracycline, mg/kg				
Days Withdrawal	Muscle	Liver	Kidney	Fat	Blood
0	0.92	1.98	3.46	0.48	0.84
3	0.51	1.27	2.39	0.41	0.63
7	0.20	0.36	0.96	0.13	0.21
10	0.08	0.17	0.43	0.06	0.08

Since all calves showed measurable residues of tetracycline after a ten-day withdrawal period on the above experiment, another study was conducted with Holstein calves (average weight 54.3 kg) to study longer withdrawal times (Eggert, 1978b). The calves were given tetracycline oral boluses to provide 22 mg/kg bw daily for ten consecutive days. The calves were fed a nonmedicated milk replacer twice daily, plus a commercial nonmedicated calf starter grain mix *ad libitum*. Three calves each were sacrificed at 0, 10, 14, 17, 20 and 24 days after cessation of tetracycline treatments for microbiological assay of residues in blood and tissues. The results of these assays are shown in Table 29.

Table 29. **Average Residues of Tetracycline in Blood and Tissues Samples from Calves Following Cessation of Treatment with Tetracycline Boluses Furnishing 22 mg/kg Daily for 10 Days**

	Tetracycline, mg/kg				
Days Withdrawal	**Muscle**	**Liver**	**Kidney**	**Fat**	**Blood**
0	0.27	0.90	1.65	0.17	0.36
10	ND[1]	ND	ND-0.09	ND	ND
14	ND	ND	ND	ND	ND
17	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND
24	ND	ND	ND	ND	ND

[1] ND = Not Detected, less than sensitivity of microbiological assay method, 0.0375 mg/L for blood and 0.1 mg/kg for tissues

The results of this experiment differed from those obtained in the earlier experiment in two respects. First the residue levels found at zero day-withdrawal in the present experiment were lower for all tissues than for the earlier experiment. Secondly, no detectable residues were found in any of the tissues taken at 10-day withdrawal with the exception of one kidney sample which showed an apparent residue of 0.09 ppm (which is less than the validated limit of the assay method), while in the earlier experiment all tissue samples taken at the 10-day withdrawal period showed positive residues. The only difference in the procedures used in the two experiments is that in the second experiment a commercial calf starter gratin ration was fed in addition to the milk replacer diet. This difference in feeding management may be responsible for the disparity between the two experiments. Bradley et al. (1982) found in studies with calves given oral doses of chlortetracycline that milk fed calves absorbed a larger fraction of the chlortetracycline dose, had a smaller volume of distribution and a smaller overall body clearance rate than did conventionally fed calves. A similar effect might be anticipated with tetracycline.

Depletion of Residues from Edible Tissues of Chickens

A study was conducted in which broiler chickens were given drinking water containing 2830 mg tetracycline bydrochloride/gallon of water for five consecutive days. Tetracycline residues were measured by microbiological assays for blood and tissues during medication and after cessation of treatment (Hewell, 1987). The results of this study are summarised in Table 30.

Depletion of Residues from Eggs

Roudaut, et al (1989) treated laying hens for five consecutive days with drinking water containing 250 and 500 mg tetracycline/litre and for seven consecutive days with feed containing 300 and 600 ppm of tetracycline. Residues were measured separately in the yolk and albumin fractions of the egg. A summary of the residue data, measured by microbiological assay, is shown in Tables 31 and 32.

Table 30. **Average Tetracycline Residues in Blood and Tissues of Broiler Chickens During and After Supply with Drinking Water Containing 2830 mg/gallon of Tetracycline for Five Days**

	Tetracycline, mg/kg				
During Treatment	Sex	Blood	Muscle	Liver	Kidney
0 hour	Male	ND[1]	NM[2]	NM	NM
	Female	ND	NM	NM	NM
6 hours	Male	0.773	NM	NM	NM
	Female	0.590	NM	NM	NM
12 hours	Male	0.765	NM	NM	NM
	Female	0.214	NM	NM	NM
24 hours	Male	0.383	0.793	1.870	6.225
	Female	0.702	0.908	2.380	6.450
36 hours	Male	0.649	NM	NM	NM
	Female	0.704	NM	NM	NM
After Treatment					
0 hour	Male	0.433	0.821	1.845	6.113
	Female	0.578	0.978	1.973	6.210
12 hours	Male	0.083	0.198	0.368	1.450
	Female	ND	ND-0.118	ND-0.175	0.681
24 hours	Male	ND-0.068	0.140	0.232	1.390
	Female	ND-0.042	ND-0.099	ND-0.150	0.605
48 hours	Male	ND	NM	NM	NM
	Female	ND	NM	NM	NM

[1] ND = Not Detected, less than sensitivity of method, 0.0375 mg/L for blood and 0.1 mg/kg for tissues; [2] NM = Not Measured

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Table 31. **Average Tetracycline Residues (±SD) in Albumin and Yolk Fractions of the Egg and the Whole Eggs Following a Five Day Treatment with 250 and 500 mg/L Tetracycline in Water**

	Tetracycline, mg/kg					
	Albumin		Yolk		Whole Egg	
Dose	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm
Day of treatment						
5	0.11±0.03	0.19±0.07	0.52±0.19	0.81±0.44	0.25±0.08	0.40±0.19
Withdrawal Day						
1	0.10±0.03	0.17±0.03	0.52±0.14	1.02±0.52	0.25±0.07	0.44±0.19
2	<0.07	0.07±0.02	0.46±0.13	1.11±0.48	0.17±0.05	0.40±0.17
3		<0.07	0.42±0.12	0.91±0.46	0.17±0.06	0.29±0.15
4			0.31±0.10	0.80±0.37	0.11±0.04	0.27±0.14
5			0.22±0.08	0.53±0.19	0.08±0.03	0.18±0.07
6			0.20±0.07	0.40±0.17	0.07±0.02	0.12±0.06
7			<0.15	0.32±0.18		0.10±0.06
8				0.21±0.16		0.07±0.05
9				0.19±0.15		0.06±0.05
10				<0.15		

Table 32. **Average Tetracycline Residues (±SD) in Albumin and Yolk Fractions of the Egg and the Whole Eggs Following a Seven Day Treatment with 300 and 600 ppm of Tetracycline in Feed**

	Tetracycline, mg/kg					
	Albumin		Yolk		Whole Egg	
	300 ppm	600 ppm	300 ppm	600 ppm	300 ppm	600 ppm
Day of Treatment						
7	0.19±0.02	0.30±0.08	0.87±0.07	1.41±0.40	0.41±0.04	0.61±0.15
Withdrawal Day						
1	0.12±0.03	0.20±0.06	0.98±0.09	1.51±0.45	0.41±0.05	0.60±0.15
2	<0.07	0.08±0.02	1.12±0.18	1.58±0.46	0.40±0.07	0.54±0.15
3		<0.07	0.78±0.18	1.10±0.25	0.27±0.07	0.37±0.08
4			0.63±0.15	0.94±0.30	0.21±0.06	0.31±0.09
5			0.49±0.15	0.64±0.19	0.16±0.05	0.22±0.06
6			0.31±0.06	0.48±0.17	0.10±0.02	0.15±0.06
7			0.27±0.06	0.39±0.15	0.09±0.02	0.13±0.05
8			0.20±0.04	0.37±0.15	0.07±0.02	0.11±0.04
9			<0.15	0.28±0.11		0.09±0.03
10				0.24±0.08		0.07±0.02
11				0.18±0.11		0.05±0.03
12				<0.15		

Residues in Milk

Tetracycline is generally little used for intramammary infusion for control of mastitis because it tends to cause too much tissue irritation. However tetracycline residues can result from other therapeutic uses of tetracycline in lactating animals. A study was conducted using six lactating dairy cows which had two 500-mg tetracycline boluses placed in the uterus two to six days following parturition (Brown, 1961). Milk samples were taken from each cow prior to treatment and at 12-hour intervals following treatment. Microbiological assays showed no antibiotic activity in any of the pretreatment samples. The results of the assays of the post treatment samples is shown in Table 33.

Table 33. **Residues (mg/L) in Milk from Cows Following Intrauterine Treatment with 1000 mg of Tetracycline in Bolus Form**

	Tetracycline, mg/L					
Hours Postmedication	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6
12	0.26	0.14	0.28	0.13	0.06	0.39
24	0.22	0.08	0.23	0.05	0.06	0.41
36	0.16	0.13	0.14	0.20	0.12	0.36
48	0.14	0.05	0.12	0.05	0.11	0.28
60	ND[1]	ND	ND	ND	ND	0.25
72	ND	ND	ND	ND	ND	0.11
84	ND	ND	ND	ND	ND	ND
96	ND	ND	ND	ND	ND	ND

[1] ND = Not Detected (LOD not given)

Another study was conducted in which the Delvo-test P was used to check milk samples for antibiotic residues (Haaland et al, 1984). In this test, a solution of tetracycline was infused into the uterus, generally as a treatment for retained placenta, but also for other reasons. Milk samples were tested from each cow at 12 hour intervals following treatment until the milk was clear of all detectable antibiotic activity. The results obtained for cows treated with tetracycline are shown in Table 34.

Table 34. **Results with Cows Given Intrauterine Infusions of Tetracycline for Treatment of Retained Placenta or Other Uterine Infections.**

Tetracycline Treatment	No. of Cows	Hours to Clear Antibiotic Residues
1 infusion of 3,000 mg	9	72-84
1 infusion of 500 mg	4	60
3 infusion of 500 mg	4	72-84

The nine cows receiving the single infusions of 3,000 mg of tetracycline for retained placenta required 72 to 84 hours to clear antibiotic residues from the milk. More than 60 hours were required for the milk to clear in 4 cows treated once with a 500-mg dose. Three treatments, each containing 500 mg of tetracycline given at a 24-hour interval, resulted in persistence of milk residues for the same period of time as one 3,000-mg infusion.

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METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Analytical procedures for tetracyclines based on both microbiological and chemical assays have been discussed earlier (see monograph on Chlortetracycline in this volume). In general, chemical and microbiological methods measure the three commonly used tetracyclines, tetracycline, oxytetracycline and chlortetracycline together as a group. The chemical and microbiological procedure give similar quantitative results for the same antibiotic with the modern chemical testing having at least a ten fold sensitivity advantage while being less cost effective.

APPRAISAL

Tetracycline was last evaluated by JEFCA at the 12th Meeting in 1968, together with oxytetracycline and chlortetracycline. At that time, for tetracycline maximum residue levels were recommended of 0.5 mg/kg in meat, 0.3 mg/kg in eggs and 0.1 mg/kg in milk, calculated as base.

The predominant use of tetracycline is as a therapeutic drug. It is rapidly but only moderately well absorbed from the GI tract and is eliminated in both urine and faeces either unchanged or in a microbiologically inactive form. There is no evidence that tetracycline is significantly metabolised *in vivo* although some isomerisation of the drug can occur, either in the animal and/or during isolation. Microbiological assay is therefore a satisfactory method to acquire depletion of tetracycline from tissues. Tissue depletion measurements conducted both by microbiological and chemical assay give very similar results. Differences in microbiological potency of individual tetracyclines, however, requires a preference for chemical determination of residues for regulatory purposes. Since tetracycline undergoes minimal metabolism, it is the appropriate marker compound for determination of residues in tissues.

Serum level and residue studies indicate that tetracycline is both rapidly absorbed and quickly cleared from edible tissues following oral administration. Kidney and liver tissues in all species show the largest concentration of tetracycline both at the withdrawal from medication, and at any time point during the withdrawal period. When liver and kidney tissues are in compliance with any designated MRL. residues in muscle will be less than 10% those found in kidney and fat should not cause any problem. Either liver or kidney (or both) could be considered as target tissue. It is recommended that kidney is the preferred target tissue.

In pigs given tetracycline at 24 mg per kg body weight per day for 14 days in drinking-water, mean residue levels were 0.2 and 0.1 mg/kg in kidney and liver, respectively, 4 days after withdrawal of medication. Calves on the same dose had average residue levels of 0.4 and 0.17 mg/kg in kidney and liver, respectively, 10 days after withdrawal of medication. In chickens given 620 mg/L of tetracycline in drinking water for 5 days, average residue levels in kidney and liver were 1.4 and 0.2 mg/kg, respectively, 24 hours after withdrawal of the drug, while residues in whole egg averaged 0.27 mg/kg at 4 days withdrawal, declining to <0.06 mg/kg 10 days after withdrawal. A dose of 3 g given to a lactating cow by intrauterine infusion led to residues of <0.10 mg/kg in milk 84 hours after dosing.

Maximum Residue Limits

In reaching its decision on MRLs for tetracycline (and chlortetracycline) the Committee considered the following:

- MRLs were recommended for oxytetracycline at the 36th meeting of the Committee for all species of 600 µg/kg in kidney, 300 µg/kg in liver, 100 µg/kg in muscle, 100 µg/kg in milk, 200 µg/kg in eggs, and 10 µg/kg in fat. These levels were the lowest detectable by validated antimicrobial methods;

- chlortetracycline and tetracycline have been allocated a group ADI of 0-3 µg per kg of body weight with oxytetracycline;

- modern analytical techniques allow much more sensitive and specific assays than those provided by antimicrobial assays;

- the recommended target tissues for residue analysis in cattle, pigs and poultry are kidney and muscle. Based on limited data, the kidney is the recommeoded target tissue for sheep; and

- the marker residue for all three substances is parent drug.

The Committee recommended the following temporary MRLs for (both) tetracycline (and chlortetracycline) in cattle, pigs and poultry, expressed as parent drug:

Muscle - 100 µg/kg
Liver - 300 µg/kg
Kidney - 600 µg/kg
Eggs (poultry) - 200 µg/kg

The Committee also recommended temporary MRLs for sheep liver and kidney of 300 µg/kg and 600 µg/kg, respectively, expressed as parent drug.

The following information is required for evaluation in 1996:

- The results of residue depletion studies in cattle, sheep, pigs and poultry to determine the rate of depletion of residues in milk (cows), fat (all species) and in muscle, liver, kidney and fat (sheep), treated in accordance with approved uses of these substances; and

- new and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in tissues and milk.

ADI and MRLs allocated to chlortetracycline, and tetracycline are the same as those previously allocated to oxytetracycline at the 36th meeting for the given tissues and species. Although the Committee realized that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to tetracyclines were defined as applying both to residues of individual tetracyclines and to the sum of combined tetracycline residues, including chlortetracycline, oxytetracycline and tetracycline.

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ANNEX 1

SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

The attached table summarizes the veterinary drug evaluations conducted by JECFA at the 32nd (1987), the 34th (1989), the 36th (1990), the 38th (1991), the 40th (1992), the 42nd (1994), the 43rd (1994), and 45th (1995) Meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in foods. Please see Reports of those meetings, published in WHO Technical Report Series.

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (TE).
- Where an MRL is temporary, it is so indicated by "TE".
- Several compounds have been evaluated more than once. The data given is for the most recent evaluation.

Substance	ADI (μg/kg bw)	ADI status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue (MR)
Abamectin	0-0.2[1]	Full	45 (1995)	No MRL			
Albendazole	0-50	Full	34 (1989)	100[2] 5000[2]	Muscle, fat, milk Liver, kidney	Cattle, sheep	2-Aminobenzimidazole sulfone, MR in milk needs to be identified
Azaperone	0-3	TE	43 (1994)	60 TE 100 TE	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol

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[1] The ADI for abamectin was established by the 1994 Joint Meeting on Pesticide Residues (JMPR)

[2] Parent drug equivalents

Benzylpenicillin	30 μg/person/day	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
BST	Not specified	Full	40 (1992)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	
Carbadox	Limited acceptance	Full	36 (1990)	30[1] 5[1]	Liver Muscle	Pigs	Quinoxaline-2-carboxylic acid
Carazolol	0-0.1	Full	43 (1994)	5[2] 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug
Ceftiofur sodium	0-50	Full	45 (1995)	200 2000 4000 600 100 μg/l	Muscle Liver Kidney Fat Milk	Cattle, pigs Cattle	Desfuroylceftiofur
Chloramphenicol	None	No	42 (1994)	No MRL			
Chlorpromazine	None	No	38 (1991)	No MRL			
Chlortetracycline & tetracycline	0-3[3]	Full	45 (1995)	100 TE 300 TE 600 TE 200 TE	Muscle Liver Kidney Eggs	Cattle, pigs & poultry Cattle, pigs, sheep & poultry Poultry	Parent drug

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[1] As quinoxaline-2-carboxylic acid (QCA)

[2] The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effects of carazolol

[3] Group ADI for chlortetracycline, oxytetracycline and tetracycline

Closantel	0-30	Full	36 (1990) 40 (1990)	1000 3000 1500 5000 2000	Muscle, liver Kidney, fat Muscle, liver Kidney Fat	Cattle Sheep	Parent drug
Dexamethasone	0-0.015	Full	42 (1992) 43 (1994)	0.5 TE 2.5 TE 0.3 μg/l TE	Muscle, kidney Liver Milk	Cattle, horses, pigs Cattle	Parent drug
Diclazuril	0-20	TE	45 (1995)	500 TE 3000 TE 2000 TE 1000 TE	Muscle Liver Kidney Fat	Sheep, rabbits & poultry	Parent drug
Dihydrostreptomycin & streptomycin	0-30 (Group ADI)	TE	43 (1994)	500 TE 1000 TE 200 μg/l TE	Muscle, liver, fat Kidney Milk	Cattle, pigs, chickens, sheep Cattle	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	None	No	34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 μg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Doramectin	0-0.5	Full	45 (1995)	10[1] 100 30 150	Muscle Liver Kidney Fat	Cattle	Parent drug
Enrofloxacin	0-0.6	TE	43 (1994)	No MRL			
Estradiol-17β	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	

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[1] The Committee noted the high concentration of residues at the injection site during the 35-day period after parenteral administration of the recommended dose

Febantel	0-4[1]	TE	45 (1995)	100 TE	Muscle, kidney, fat	Cattle, sheep, pigs	Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents
				500 TE	Liver		
				100 $\mu g/l$ TE	Milk	Cattle	
Fenbendazole (see febantel)							
Flubendazole	0-12	Full	40 (1992)	10	Muscle, liver	Pigs	Parent drug
				200	Muscle	Poultry	
				500	Liver		
				400	Eggs		
Flumequine	None	No	42 (1994)	No MRL			
Furazolidone	None	No	40 (1992)	No MRL			
Gentamicin	0-4	TE	43 (1994)	100 TE	Muscle, fat	Cattle, pigs	Parent drug
				200 TE	Liver		
				1000 TE	Kidney		
				100 $\mu g/l$ TE	Milk	Cattle	
Ipronidazole	None	No	34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100	Muscle, fat, milk	Cattle	Parent drug
				500	Liver		
				1000	Kidney		
Ivermectin	0-1	Full	40 (1992)	100	Liver	Cattle	H_2B_{1a}
				40	Fat		
			36 (1990)	15	Liver	Other species	
				20	Fat		
Levamisole	0-6	Full	42 (1992)	10	Muscle, kidney, fat	Cattle, sheep, pigs and poultry	Parent drug
				100	Liver		

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[1] Group temporary ADI for febantel, fenbendazole and oxfendazole

Metronidazole	None	No	34 (1989)	No MRL			
Moxidectin	0-2	Full	45 (1995)	20[1] 100 50 500	Muscle Liver Kidney Fat	Cattle, sheep & deer[2]	Parent drug
Neomycin	0-30	TE	43 (1994)	500 TE 5000 TE 500 TE 500 μg/l TE	Muscle, liver, fat Kidney Eggs Milk	Cattle, chickens, ducks, goats, pigs, sheep, turkeys Chickens Cattle	
Nitrofurazone	None	No	40 (1992)	No MRL			
Olaquindox	Limited acceptance	TE	42 (1994)	No MRL but 4 μg/kg of MQCA (TE) is consistent with GVP	Muscle	Pigs	MQCA
Oxfendazole (see febantel)							
Oxolinic acid	None	No	43 (1994)	No MRL			

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[1] The Committee noted the very high concentration of residues at the injection site during the 35-day period after parenteral administration of the recommended dose

[2] Temporary

Oxytetracycline	0-3[1]	Full	36 (1990)	100	Muscle	All species	Parent drug
				300	Liver		
				600	Kidney		
				10	Fat		
				100	Milk		
				200	Eggs		
			45 (1995)	100 TE	Giant prawn	*Penaeus monodon*	
Progesterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Propionylpromazine	None	No	38 (1991)	No MRL			
Ractopamine	None	No	40 (1992)	No MRL			
Ronidazole	Withdrawn	No	42 (1994)	No MRL			
Spectinomycin	0-40	Full	42 (1994)	300 TE	Muscle	Cattle, pigs, and chicken	Parent drug
				2000 TE	Liver		
				5000 TE	Kidney		
				500 TE	Fat		
				200 $\mu g/l$ TE	Milk		
Spiramycin	0-50	Full	43 (1994)	100[*]	Muscle	Cattle	[*]Sum of spiramycin and neospiramycin
				200[**]	Muscle	Pigs	
				200[*]	Muscle	Chickens	
				300[*]	Liver	Cattle	[**]Expressed as spiramycin equivalents (antimicrobially active residues)
				600 TE[**]	Liver	Pigs	
				400[*]	Liver	Chickens	
				200[*]	Kidney	Cattle	
				300 TE[**]	Kidney	Pigs	
				800[*]	Kidney	Chickens	
				300[*]	Fat	Cattle	
				200 TE[**]	Fat	Pigs	
				300[*]	Fat	Chickens	
				100 $\mu g/l$[*]	Milk	Cattle	

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[1] Group ADI for chlortetracycline, oxytetracycline and tetracycline

Streptomycin (see dihydrostreptomycin)							
Sulfadimidine	0-50	Full	42 (1994)	100 25 $\mu g/l$	Muscle, liver, kidney, fat Milk	Cattle, sheep, pigs and poultry Cattle	Parent drug
Sulphthiazole	None	No	34 (1989)	No MRL			
Testosterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Tetracycline (see chlortetracycline							
Tiabendazole	0-100	Full	40 (1992)	100 100	Muscle, liver, kidney, fat Milk	Cattle, pigs, goats and sheep Cattle, goats	Sum of tiabendazole and 5-hydroxytiabendazole
Trenbolone acetate	0-0.02	Full	34 (1989)	2 as MR 10 as MR	Muscle Liver	Cattle	β-Trenbolone α-Trenbolone
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver, kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one
Tylosin	None	No	38 (1991)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Parent drug

RECOMMENDATIONS ON COMPOUNDS EVALUATED BY THE 45TH JECFA

Substance	ADI µg/kg bw	MRLs
Anthelminthic agents		
Abamectin	0-0.2[a]	No MRLs recommended[b]
Doramectin	0-0.5	Muscle (cattle): 10 µg/kg[c,d] Liver (cattle): 100 µg/kg[c,d] Kidney (cattle): 30 µg/kg[c,d] Fat (cattle): 150 µg/kg[c,d]
Moxidectin	0-2	Muscle (cattle, sheep & deer[e]): 20 µg/kg[c,f] Liver (cattle, sheep & deer[e]): 100 µg/kg[c,f] Kidney (cattle, sheep & deer[e]): 50 µg/kg[c,f] Fat (cattle, sheep & deer[e]): 500 µg/kg[c,f]
Febantel, fenbendazole and oxfendazole	0-4[g]	Muscle, kidney & fat (cattle, pigs & sheep): 100 µg/kg[h,i] Liver (cattle, pigs & sheep): 500 µg/kg[h,i] Milk (cattle & sheep): 100 µg/l[h,i]
Antimicrobial agents		
Ceftiofur sodium	0-50	Muscle (cattle, pigs): 200 µg/kg[j] Liver (cattle & pigs): 2000 µg/kg[j] Kidney (cattle & pigs): 4000 µg/kg[j] Fat (cattle & pigs): 600 µg/kg[j] Milk (cattle): 100 µg/l[j]
Chlortetracycline and tetracycline	0-3[k]	Muscle (cattle, pigs & poultry): 100 µg/kg[c,h] Liver (cattle, pigs, sheep & poultry): 300 µg/kg[c,h] Kidney (cattle, pigs, sheep & poultry): 600 µg/kg[c,h] Eggs (poultry): 200 µg/kg[c,h]
Oxytetracycline	0-3[k]	Giant prawn (*Penaeus monodon*): 100 µg/kg[c,h]
Antiprotozoal agent		
Diclazuril	0-20[l]	Muscle (sheep, rabbits & poultry): 500 µg/kg[c,m] Liver (sheep, rabbits & poultry): 3000 µg/kg[c,m] Kidney (sheep, rabbits & poultry): 2000 µg/kg[c,m] Fat (sheep, rabbits & poultry): 1000 µg/kg[c,m]

NOTES

a. The ADI for abamectin was established by the 1994 Joint FAO/WHO Meeting on Pesticide Residues (JMPR; Report: FAO Plant Production and Protection Paper 127, 1995; Toxicological monograph: WHO/PCS/95.2, 1995).

b. Several issues relating to differences in approaches used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and JMPR for recommending MRLs in animal products arose at the meeting. In addition, the ADI established by JMPR for abamectin used an increased safety factor to take into account the Δ-8,9 isomer, a metabolite that is not present in animal products when the substance is used as a veterinary drug. Therefore, the Committee did not recommend MRLs for abamectin used as veterinary drug and recommended that consultations be held between representatives of JECFA and JMPR to resolve the ADI and MRL issues.

c. Expressed as parent drug.

d. The Committee noted the high concentration of residues at the injection site during the 35-day period after parenteral administration of the recommended dose.

e. Temporary MRL for deer.

f. The Committee noted very high concentrations and great variation in the level of residues at the injection site over a 49-day period after dosing cattle.

g. Group temporary ADI for febantel, fenbendazole and oxfendazole, based on the no-observed-effect level (NOEL) for oxfendazole identified at the thirty-eight meeting of the Committee (WHO Technical Report Series, No. 815, 1991).

h. Temporary MRL.

i. Determined as the sum of fenbendazole, oxfendazole and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents.

j. Expressed as desfuroylceftiofur.

k. Group ADI for chlortetracycline, oxytetracycline and tetracycline, based on the NOEL for oxytetracycline identified at the thirty-sixth meeting of the Committee (WHO Technical Report Series, No. 799, 1990).

l. Temporary ADI.

m. MRL is temporary because ADI is temporary.

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1/2 Review of food consumption surveys 1977 – Vol. 2. Africa, Latin America, Near East, Far East, 1979 (E)
2 Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E F S)
3 Report of a joint FAO/WHO expert consultation on dietary fats and oils in human nutrition, 1977 (E F S)
4 JECFA specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers, 1978 (E)
5 JECFA – guide to specifications, 1978 (E F)
5 Rev. 1 JECFA – guide to specifications, 1983 (E F)
5 Rev. 2 JECFA – guide to specifications, 1991 (E)
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14/1 Rev.1 The food control laboratory, 1986 (E)
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